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THE EFFECT OF HYDROCARBON CONTAMINATION AND SUBSTRATE
MATERIAL ON OYSTER REEF COMMENSAL COMMUNITIES

A Thesis

Submitted to Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Biological Sciences

by

Jenessa Lea Kay

B.A. University of North Carolina at Chapel Hill, 2010

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ABSTRACT

Oyster reefs in the Gulf of Mexico provide water quality enhancement, shoreline stabilization, carbon sequestration, and facilitate spat recruitment. They are also essential refuges for numerous resident fish and invertebrates, in turn supporting commercial fisheries. Oyster reefs are however in danger worldwide as oyster fisheries increase and pollution from oil spills, such as the *Deepwater Horizon* spill, further degrade reefs. The development of artificial reefs has therefore become a necessity. This study assesses both the long-term and acute response of oyster reef commensal communities to hydrocarbon contamination, as well as comparing the efficacy of artificial reef substrates for restoring these faunal assemblages. Long-term effects were analyzed by quantifying commensal abundance, taxa richness, and diversity from cultch-filled bags deployed at two sites in Barataria Bay, Louisiana, that experienced oiling from *Deepwater Horizon*, and two control sites. Bags were deployed seasonally in both 2012 and 2013, and the results indicated that while commensal abundance was generally greater at oiled sites, the effects of hydrocarbon contamination several years post spill were neither large nor consistent. To observe the acute colonization response, oil-soaked and control bags were retrieved 1, 2, 4, and 8 weeks after deployment at Grand Isle, LA, an area in Barataria Bay where no oil contamination was documented, in both June and September 2013. Oil effects on commensal communities were inconsistent and minimal by week 8, perhaps due to biodegradation of the hydrocarbons. Commensal communities were also sampled from bags containing either disarticulated oyster shell, limestone rubble or a composite material known as OysterCrete. While OysterCrete had the greatest abundance of commensal organisms, the experiments indicated that seasonal variation was more influential for commensal community dynamics, as well as new spat recruitment and growth, than the presence of hydrocarbons or

various substrates. In areas in close proximity to major oil operations, such as the northern Gulf of Mexico, any restoration efforts that provide a hard substrate will be beneficial for the recruitment of commensal organisms if natural oyster reefs are impacted by anthropogenic disturbances.

INTRODUCTION

Eastern Oyster (*Crassostrea virginica*) reefs are one of the most economically and environmentally productive ecosystems in the southeastern United States. The Gulf of Mexico produces nearly two-thirds of the country's oyster harvest by volume and over one-half by value, with Louisiana accounting for one-third of this production (Louisiana Department of Wildlife and Fisheries, LDWF 2012). The economic importance of oysters also includes the creation of numerous jobs, ranging from oyster farming, harvest, processing and selling, to artificial reef construction projects.

In addition to having significant economic and cultural importance to the Gulf of Mexico states, oyster reefs also provide many valuable ecosystem services. These services include improving water quality, stabilizing the shoreline, and the creation of essential habitat. By filtering excess nutrients, oysters mitigate the harmful effects of eutrophication as a result of anthropogenic nutrient loading (Jackson et al. 2001; Cerco and Noel 2007; Newell et al. 2007). By filtering sediment out of the water column, oysters also improve water clarity and light attenuation, which in turn supports primary productivity in coastal habitats such as salt marshes and submerged seagrass beds, both of which are important sources of food and habitat for many marine organisms (Meyer et al. 1997; Heck et al. 2003). Other environmentally important ecosystem services provided by oysters include the denitrification of coastal waters (Piehler and Smyth 2011) and the sequestration of carbon into their calcium carbonate shell matrices (Hargis et al. 1999; Peterson and Lipcius 2003).

The gregarious, reef-forming nature of oysters (Cole and Knight-Jones 1939; Hidu 1969) also leads to the provision of additional ecosystem services. The three-dimensional structure created by these ecosystem engineers contributes to shoreline protection and erosion control

(Meyer et al. 1997; Piazza et al. 2005), and provides habitat and refugia from predation for many juvenile and adult species of commercially important fish and invertebrates (Kennedy 1996; Harding and Mann 1999; Posey et al. 1999). Oyster reefs also serve as important nursery grounds (Beck et al. 2001; Coen et al. 2007) for many species of nekton, and the vertical habitat complexity further enhances the biodiversity of taxa supported by the reef ecosystem (Wells 1961; Meyer and Townsend 2000; Soniat et al. 2004). The presence of these nekton and macroinvertebrates has been shown to directly enhance the production of other economically important fisheries (Coen et al. 1999; Harding and Mann 2001; Tolley and Volety 2005).

Despite their important economic value and ecological functions, up to 85% of oyster reefs have declined from their historical abundances globally (Beck et al. 2011). This can largely be attributed to destructive harvest techniques (Lenihan and Micheli 2000; Kirby 2004), changes in sedimentation regimes (Smith et al. 1997), and increased prevalence of diseases such as *Perkinsus marinus* or *MSX*, and hypoxia (Lenihan and Peterson 1998).

While Beck et al. (2011) reported that oysters in the Gulf of Mexico are in “fair” condition compared to other regions of the United States, 2010 Louisiana oyster landings were the lowest documented since 1966 (LDWF 2012; Lutz et al. 2012). Many speculate that this apparent decline in oyster abundance was directly related to the *Deepwater Horizon (DWH)* oil spill which occurred in April 2010, as the toxicological effects of hydrocarbons on marine fish and filter-feeding invertebrates have been well documented (Neff and Anderson, 1981; Peterson 2001). In addition to oiled substrates negatively impacting organismal development and recruitment, marine fauna also assimilate pollution from the water column into their tissues via their gills, often resulting in carcinogenic and mutagenic effects (Lehr and Jerina 1977; Baumard et al. 1999).

The *DWH* oil spill released approximately 4.9 million barrels of Light South Crude Louisiana Oil into the Gulf of Mexico over the 87 day period that the Macondo wellhead remained open (Crone and Tolstoy 2010). In May 2010, oil reached coastal Louisiana and eventually 125 miles of Louisiana's coastline received some degree of contamination (Klemas 2010). Oyster reefs were also closed to harvesting for a significant amount of time in 2010. Reduced salinity on many of Louisiana's oyster reefs impacted by the *DWH* oil spill also likely contributed to oyster mortality in those areas.

Understanding the potential devastation from oil spills such as *DWH* is of critical importance, especially in the Gulf of Mexico where the likelihood of contamination is high yet the nature of oil spills is unpredictable. Past spills, such as *Exxon Valdez* in Prince William Sound, Alaska and the 1986 Panamanian oil spill, have resulted in immediate and large-scale mortalities, which also included the loss of essential ecosystem services (Jackson et al. 1989; Peterson et al. 2003b; Silliman et al. 2012). Previous research has also indicated that due to the large volume of water they filter, oysters (and, hence, the ecosystem services they provide) are especially at risk from polycyclic aromatic hydrocarbon (PAH) contamination (Banks and Brown 2002). One study, however, (Hulathduwa and Brown 2006) has indicated that other environmental variables, such as changes in salinity regimes, may have a greater effect on the abundance and distribution of oyster commensal assemblages than does hydrocarbon contamination, and further investigation is therefore necessary to fully understand the impact and recovery response of these organisms to wide-scale oil disturbances.

Artificial reefs have proven to be a successful method for the restoration or enhancement of oyster reefs (Meyer and Townsend 2000; Powers et al. 2009) and may prove to be an essential tool for recovery following *DWH* or similar catastrophes. Recently, artificial reefs have been

utilized to restore lost ecosystem services, such as shoreline stabilization (Campbell 2004; Piazza et al. 2005; Scyphers et al. 2011), improving local water quality (Nelson et al. 2004), and commercial fishery stock enhancement (Peterson et al. 2003a), in addition to their historical purpose of replenishing depleted oyster stocks (Grabowski and Peterson 2007; Brown et al. 2014). It has been estimated that the value of one hectare of restored oyster reef accounts for up to \$100,000 in ecosystem services annually (Grabowski et al. 2012).

Despite this recent phase shift towards restoring lost ecosystem services, little attention has been directed towards conservation of the commensal assemblages associated with oyster reefs in the Gulf of Mexico. While no valuation has been currently estimated for these macrobenthic populations (Grabowski et al. 2012), these organisms increase the overall biodiversity of reef ecosystems and many species are important prey items for higher trophic levels, including many commercially important species; thus a need exists for restoration efforts to also target these communities. Studies have shown that macrofauna abundance has increased on restored oyster plots (Rodney and Paynter 2006); however, with most restoration efforts focusing on other aspects of oyster dynamics, the most effective artificial reef types for specifically restoring these organisms remains relatively unknown.

It is widely understood that species abundance and diversity increases with habitat complexity (MacArthur and MacArthur 1961; Heck and Wetstone 1977), and the spatial arrangement and vertical complexity of oyster reefs in particular offers excellent habitat and refuge from predation via the interstitial spaces between individual oysters (Soniati et al. 2004; Tolley and Volety 2005). Restoring reefs with native oyster cultch is often expensive and supply-limited (Soniati and Burton 2005); thus, engineering of artificial reefs often utilizes a variety of designs and materials (Meyer et al. 1997; Piazza et al. 2005; Gregalis et al. 2008).

Over 400 artificial reefs have been created in the Gulf of Mexico since 1990, nearly half of which were constructed from limestone or rock aggregate concrete (Furlong 2012; La Peyre et al. 2014). Limestone has been found to be an effective alternative to molluscan cultch for recruiting oyster veliger larvae, perhaps due to its calcium carbonate composition (Hidu et al. 1975; Chatry et al. 1986; Soniat et al. 1991). Aggregate materials often contain a biological additive, such as cottonseed, to mimic the carbonic chemical cues that recruit oyster veliger larvae (Anderson 1995; Ortega 2006). While certain invertebrates or fish may also be attracted to calcium carbonate based materials, studies have shown that structure is the most critical factor for supporting macrobenthic assemblages (Diehl 1992; Humphries et al. 2011; Brown et al. 2014), thus the need for such an additive in certain substrate materials may be erroneous with regards to commensal organisms, particularly in comparison to the refuge value provided by an increased interstitial matrix.

The purpose of this study is to examine the relative effects of hydrocarbon exposure on oyster reef commensal populations. This also examines how salinity and temporal variation interact with hydrocarbon contamination to affect these communities, as well as the recruitment of new oyster spat. In particular, both the long-term and immediate impacts following oil spill events are of primary concern. The null hypothesis is that hydrocarbon contamination has no effect on commensal abundance, richness, or diversity of commensal organisms, or on spat recruitment.

This study will also examine how different substrate types impact the colonization of oyster reefs. Mesh bags filled with oyster cultch or other commonly used reef construction materials are used to recruit commensal macrofauna populations. The null hypothesis is that there is no difference in the recruitment of commensal organisms or oyster spat between the different

substrate materials. This research could provide important implications regarding the use of artificial oyster reefs as a means of enhancing commensal communities, particularly in locations susceptible to oil contamination such as the Northern Gulf of Mexico where oyster reefs are in such close proximity to major oil production, refinery, and transport operations.

METHODS

Habitat Description

Field sites were chosen in four small bays within Barataria Bay, Louisiana (Figure 1) based on Shoreline Cleanup Assessment Technique (SCAT) Current Shoreline Oiling data from July 2010 (Figure 2; NOAA 2010), which used observational surveys to establish the magnitude of oil contamination throughout coastal Louisiana as a result of the *Deepwater Horizon* oil spill. Two oiled sites and two control sites were selected. The two oiled sites experienced “moderate” to “heavy” oil contamination while the two control sites experienced “light” or “no” oiling (Table 1). The oiled and control treatment sites were also selected so that each was either in an area of high or low salinity, as higher salinities facilitate increased oyster production (Chatry et al. 1983; Hulathduwa and Brown 2006) as well as higher rates of predation (Brown and Stickle 2002; Soniat et al. 2004).

At each sampling, temperature (°C), salinity (PSU) and dissolved oxygen (mg/L) were measured with an YSI 85 meter (Table 2). An Ekman Bottom Grab sampler was used to collect two sediment samples from each site during October of each sampling year (2011 and 2012) to analyze for tPAH (ng/g) concentrations (Table 2). Of the two control sites, Grand Isle had an average salinity of 23.7 ± 1.9 PSU in both 2012 and 2013 while Hackberry Bay had an average salinity of 12.4 ± 2.1 PSU in 2012 and 9.3 ± 1.0 PSU in 2013. Of the two oiled sites, Grand Terre had an average salinity of 23.1 ± 1.8 PSU in 2012 and 27.9 ± 1.5 PSU in 2013 while Bay Jimmy had an average salinity of 13.4 ± 2.0 PSU in 2012 and 11.6 ± 1.3 PSU in 2013.



Figure 1. A map of the four field site in Barataria Bay: Bay Jimmy, Grand Terre, Grand Isle and Hackberry Bay.

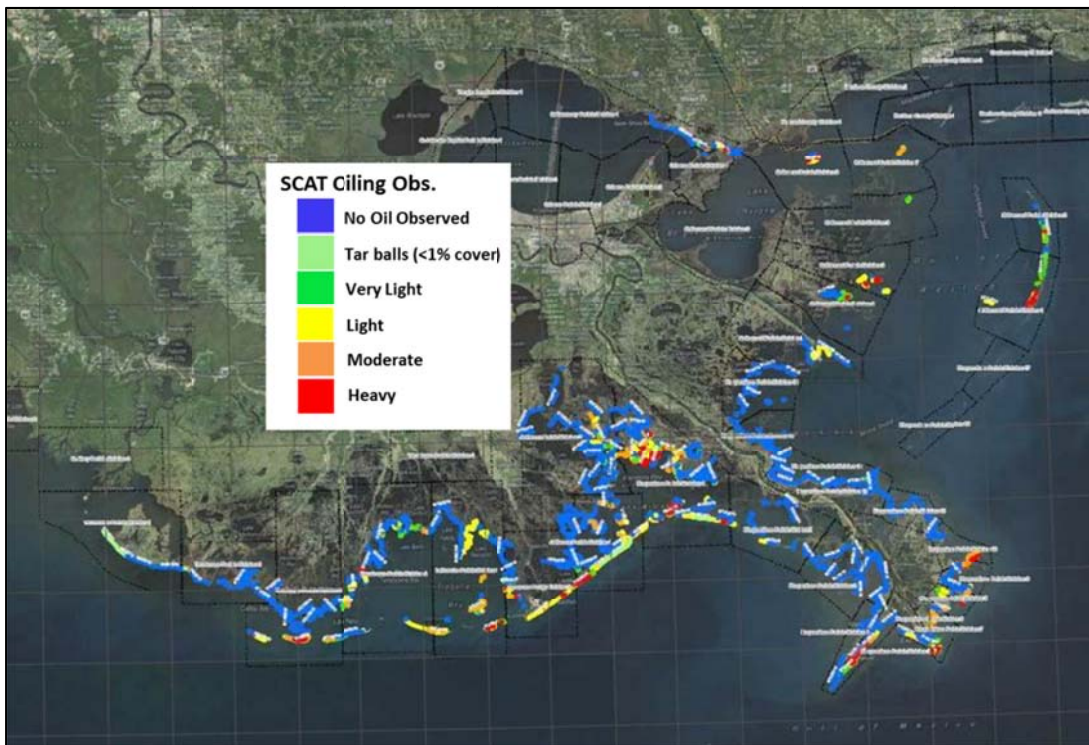


Figure 2. A map of the July 2010 Shoreline Cleanup Assessment Technique (SCAT) data used to select oiled and control treatment sites based on the magnitude of oil contamination.

Table 1. Summary of field site descriptions based on Shoreline Cleanup Assessment Technique Data and treatments (oiled, control, high and low salinity).

Site	SCAT Oil Level	Treatment	
		Oil	Salinity
Grand Isle	No Oil Observed to Very Light	Control	High
Hackberry Bay	No Oil Observed	Control	Low
Grand Terre	Light to moderate	Oiled	High
Bay Jimmy	Heavy	Oiled	Low

Table 2. Water quality data yearly means (\pm SE) for temperature ($^{\circ}$ C), salinity (PSU), dissolved oxygen (mg/L), and total polycyclic aromatic hydrocarbons (ng/g) for all field sites during sampling trips in January 2012 through November 2013.

Site	Temperature ($^{\circ}$ C)		Salinity (PSU)		DO (mg/L)		tPAH (ng/g)	
	2012	2013	2012	2013	2012	2013	2011	2012
Grand Isle	28.0 \pm 1.7	28.0 \pm 1.7	23.7 \pm 1.9	23.7 \pm 1.9	7.2 \pm 0.3	28.0 \pm 1.7	137 \pm 8.0	115 \pm 6.4
Hackberry Bay	26.9 \pm 1.5	26.8 \pm 1.1	12.4 \pm 2.1	9.3 \pm 1.0	6.7 \pm 0.5	26.8 \pm 1.1	N/A	171 \pm 13.3
Grand Terre	27.9 \pm 1.9	27.9 \pm 1.3	23.1 \pm 1.8	27.9 \pm 1.5	6.6 \pm 0.4	27.9 \pm 1.3	394 \pm 22.0	139 \pm 9.2
Bay Jimmy	26.8 \pm 1.6	27.1 \pm 1.2	13.4 \pm 2.0	11.6 \pm 1.3	6.2 \pm 0.6	27.1 \pm 1.2	519 \pm 8.8	241 \pm 14.6

Commensal Sampling

To sample the commensal communities, 0.3 m x 0.3 m bags were constructed from 2 cm Vexar \textregistered mesh and filled with clean, unbleached oyster shell. Each bag contained approximately 500 mL of shell by volume, and was attached to a PVC pole in at least 1 m of water with enough rope to allow the bag to lie on the surface of the substratum. Oyster shells ranged from

approximately 10 – 20 cm in length and were collected from seafood processors that had previously removed the meat.

To determine the long-term effects of hydrocarbon contamination on oyster commensal communities, five replicate commensal bags containing clean oyster cultch were deployed at each of the four field sites (oiled high- and low-salinity, control high- and low salinity) in Barataria Bay, LA. Bags were deployed three times per year (April, July, and September) in 2012 and 2013 to also test for seasonal differences among commensal organism assemblages. After one month, bags were carefully lifted out of the water and immediately placed in a tub to catch any loose organisms. Organisms were kept on ice for transport to the laboratory at LSU, where bags were opened and the cultch washed over a 1 mm mesh sieve. All organisms collected from the sieve were fixed in 10% formalin for at least 48 hours before being transferred to 70% ethanol for storage. Using dissecting microscopes, organisms were identified to the lowest taxonomic level possible according to an identification key by Hopkins et al. (1989), and then grouped according to taxonomic relationships (family, order or class; see Appendix) to limit bias resulting from discrepancies between identifiers (Erman 1981). For each treatment, total commensal abundance, taxa richness, and the Shannon-Weiner Diversity Index were calculated per bag.

Separate two-way ANOVAs (three sampling seasons times four sites) were conducted for each of the dependent variables (total commensal abundance, commensal taxa richness, and commensal Shannon-Weiner diversity) in each sampling year. A log transformation was used for those data which were not distributed normally according to the Shapiro-Wilks test for normality; however, only the raw data are plotted. Any significant differences between sites and sampling seasons were analyzed using Tukey's *a posteriori* tests. If the interaction term was

significant, *a posteriori* tests were conducted to compare all pairwise interactions of sites among seasons, and for each season among sites, following Underwood (1997). All statistical analyses were completed using SAS 9.4.

Acute Oil Exposure Experiment

Our sampling program started two years after the initial oil exposure following the Deepwater Horizon oil spill, some short term effects could have therefore been missed. To understand how oyster reef commensal communities respond immediately to hydrocarbon contamination on potential habitat, commensal bags containing clean or oil-soaked oyster shell were deployed at the un-oiled high-salinity site (Grand Isle, LA). Bags were deployed at this site to reduce the effects of any ambient oil in the sediment or water column that may interfere with the experiment.

Four days prior to deployment, shells were soaked in approximately 1800 mL of Louisiana “sweet” e.g. low sulfur content (Carrales and Martin 1975) crude oil with a PAH profile similar to Macondo-252 oil. Shells were soaked in 2 gallon glass jars so that the entire surface of each shell was covered in oil to simulate the heaviest oil contamination possible. The jars were sealed with Parafilm and transported to the field where the mesh bags were filled and the treatment and control bags were immediately placed in the water. Treatment bags were deployed approximately 100 meters from the unoiled control bags to prevent any cross-contamination.

Six replicate control and oil-soaked treatment bags (n=12) were retrieved 1, 2, 4, and 8 weeks post deployment. After retrieval, bags were brought back to the LSU laboratory for identification of the collected organisms. While rinsing the commensal bag shells over a 1 mm sieve, the abundance of any live oyster spat found on the shells was quantified and the sizes of ten randomly selected spat per shell piece were measured, to assess the recruitment response of

oyster spat. Bags were deployed in June 2013 and the experiment was replicated in September 2013.

The dependent variables for the commensal community were total commensal abundance, commensal taxa richness, and commensal Shannon-Weiner diversity per bag. For oyster spat settlement the dependent variables were average spat abundance and size per bag. Separate two-way ANOVAs (four retrieval intervals x treatment) were conducted for each of the dependent variables for both the June and September experiments. A log transformation was used for those data which were not distributed normally according to the Shapiro-Wilks test for normality; however, only the raw data are plotted. Any significant differences between treatments and collection time intervals were analyzed using Tukey's *a posteriori* tests. If the interactions term was significant, *a posteriori* tests were conducted to compare all pairwise interactions of sampling intervals among treatments, and for each treatment among sampling intervals (Underwood 1997). All statistical analyses were completed using SAS 9.4.

Water quality parameters (temperature (°C), salinity (PSU) and dissolved oxygen (mg/L)) were measured with an YSI 85 meter at deployment and each collection time (Table 3). Measurements were taken from both the surface and bottom waters in the immediate vicinities of both control and treatment bags, as the concentration of dissolved oxygen is linked to the rate of biodegradation of hydrocarbons in estuarine sediments (Song et al. 1986; Leahy and Colwell 1990), which is commonly oxygen-limited in the northern Gulf of Mexico (Rabalais and Turner 2001).

Table 3. Mean (\pm SE) water quality data for temperature ($^{\circ}\text{C}$), salinity (PSU), and dissolved oxygen (mg/L) for surface and bottom water for location of oil-soaked and control commensal bag deployment taken at each sampling period.

	Control		Oil	
	Surface	Bottom	Surface	Bottom
Temperature ($^{\circ}\text{C}$)	30.3 ± 0.2	30.2 ± 1.0	30.1 ± 1.5	29.8 ± 1.4
Salinity (PSU)	18.7 ± 1.1	20.3 ± 1.7	21.4 ± 2.1	21.6 ± 2.1
DO (mg/L)	8.5 ± 0.1	4.4 ± 1.4	7.7 ± 0.4	3.7 ± 1.3

Artificial Reef Substrate Material Experiment

To test for commensal community preferences for artificial reef substrate materials, 0.3 x 0.3m commensal bags were filled with one of three commonly used reef construction materials: oyster cultch, limestone rubble, and OysterCrete. The oyster cultch treatment consisted of clean, unbleached oyster shell. The limestone rubble treatment consisted of size #57 (approximately 20 mm stones) crushed limestone, obtained from a construction material supply company. The OysterCrete treatment was created in the Biological Engineering Laboratory at LSU and is composite material composed of gravel, sand, Portland cement, and a small amount of cottonseed to serve as a biological additive known to produce nitrogen similar to the natural chemical cues for spatfall emitted by adult oysters (Campbell 2004; Ortego 2006; Hall et al. 2009). The aggregate material was poured into a mold to create three-dimensional pieces approximately equivalent in size to an average oyster shell. Due to difficulties in standardizing the surface area of these materials, the mesh bags were filled based on a standardized water volume displacement of 500 mL.

Six replicate bags of each substrate treatment were attached to PVC poles and deployed in June 2013 at each of the four field sites in Barataria Bay, LA to test for commensal abundance, taxa richness, and diversity, as well for hydrocarbon and salinity effects. Bags were deployed for one month and after retrieval were brought back to the LSU laboratory for identification of the

organisms. While rinsing the commensal bag contents over a 1 mm sieve, the abundance of any live oyster spat found on the substrate materials was quantified and the sizes of ten randomly selected spat per individual substrate piece were measured to determine if there is preference between substrate materials for oyster spat settlement. The experiment was replicated in September 2013 with a sample size of five bags per substrate treatment at each site.

The dependent variables for the commensal community were total commensal abundance, commensal taxa richness, and commensal diversity per bag. For oyster spat settlement the dependent variables were average spat abundance and size per bag. Separate two-way ANOVAs were conducted for each of the dependent variables (three substrate types times four sites). A log transformation was used for that data which were not distributed normally according to the Shapiro-Wilks test for normality; however, only the raw data are plotted. Any significant differences between sites and substrate treatments were analyzed using Tukey's *a posteriori* tests. If the interactions term was significant, *a posteriori* tests were conducted to compare all pairwise interactions of substrate types among sites, and for each site among substrate types (Underwood 1997). All statistical analyses were completed using SAS 9.4.

RESULTS

Commensal Sampling

In 2012, both the time of year and site had an effect on the total abundance of organisms per bag (Table 4, Figure 3). There was also a significant month by site interaction. When comparing months within sites, there was a significant difference at the control high-salinity site between May and November ($p = 0.002$), but no significant difference between May and August or August and November. There were no significant differences between months for the control low-salinity, oiled high-salinity, or oiled low-salinity sites. There were no significant differences between sites during August, but there was a significant difference between the control low-salinity and oiled high-salinity sites ($p = 0.005$) during May. In November there was also a significant difference between the control high-salinity and oiled high-salinity sites ($p = 0.008$).

In 2013 the main effects were also significant (Table 4, Figure 3) for the log of total abundance of commensal organisms, as was the month by site interaction term (Table 4). There was a significant difference between May and November at the control high-salinity site but no significant differences between the other months. At the control-low salinity site, there was a significant difference between May and August ($p < 0.001$), May and November ($p = 0.025$), and August and November ($p = 0.001$). At the oiled high-salinity site there was a significant difference between May and November ($p = 0.002$) and between August and November ($p = 0.006$), but there was no difference between May and August. At the oiled low-salinity site there was a significant difference between May and August ($p = 0.003$) and between May and November ($p = 0.002$), but there was no difference between August and November. When comparing sites in May, all pair-wise site comparisons were significant except for between the

control low-salinity and oiled low-salinity sites. The control high-salinity and control low-salinity sites were only marginally different ($p = 0.058$). In August, there were significant differences between the control high-salinity and control low-salinity sites ($p = 0.014$) and between the control low-salinity and oiled low-salinity sites ($p = 0.002$). In November there was a significant difference between the control high-salinity and control low-salinity sites ($p < 0.001$), the control high-salinity and oiled high-salinity sites ($p = 0.002$), the control high-salinity and oiled low-salinity sites ($p < 0.001$)

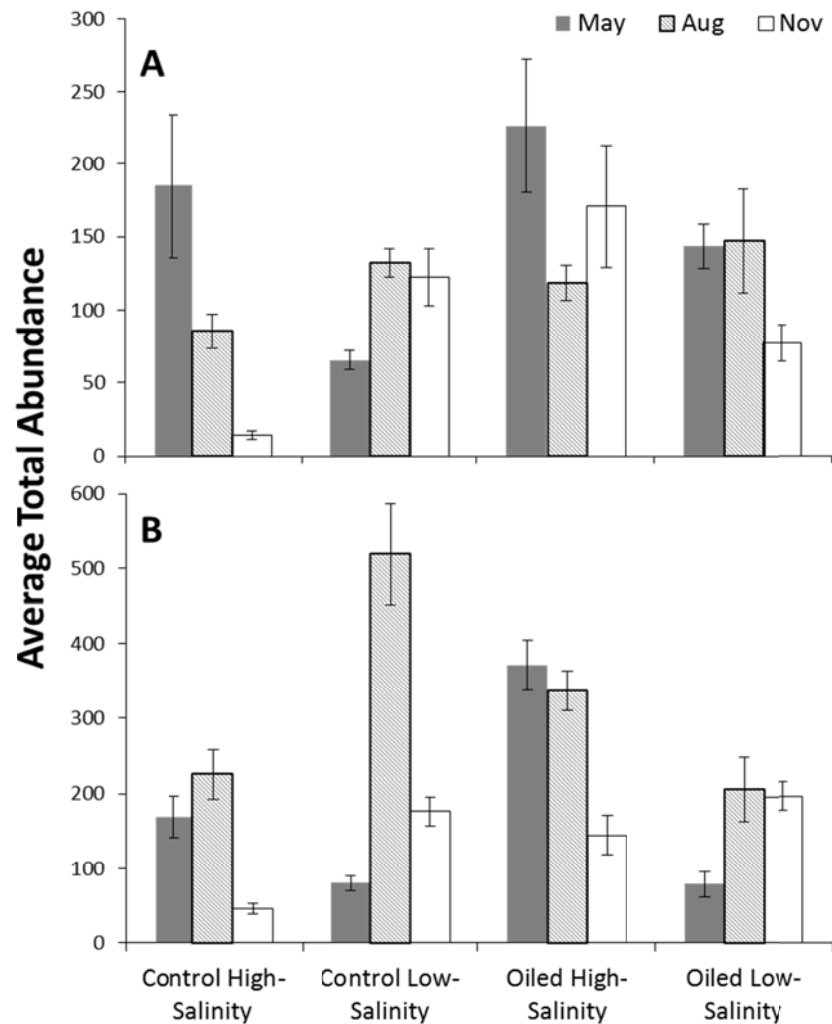


Figure 3. Mean total commensal abundance per bag \pm standard errors for at four field sites across three sampling months during (A) 2012 and (B) 2013.

In 2012, neither of the main effects were significant for taxa richness (Table 4, Figure 4). However, there was a significant month by site interaction (Table 4). When comparing sampling months within sites, the only significant difference occurred between May and November ($p = 0.003$) at the control high-salinity site. When comparing sites for May 2012, the only significant difference occurred between the control high-salinity and control low-salinity sites ($p = 0.017$). There were no significant differences among sites in August or November.

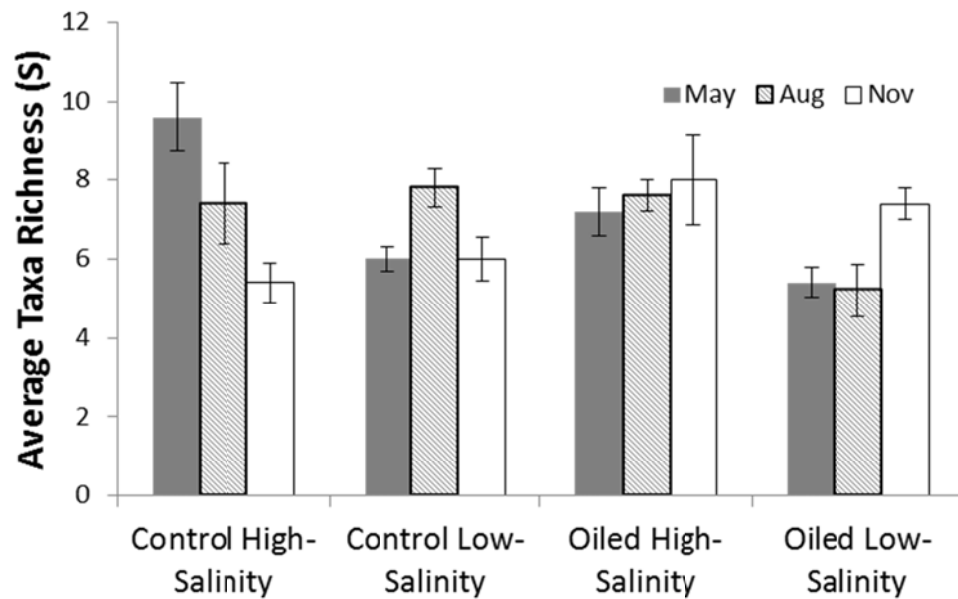


Figure 4. Mean taxa richness values \pm standard errors for at four field sites across three sampling months during 2012.

In 2013, there were significant site effects for the log of taxa richness, but there was neither a significant month effect nor a significant month by site interaction term (Table 4). Using Tukey's *a posteriori* tests to compare the main site effects (Figure 5) indicated that the control high-salinity site was significantly greater in taxa richness than the control low-salinity site ($p < 0.001$) and the oiled low-salinity site ($p < 0.001$). The oiled high-salinity site was significantly greater in taxa richness than the control low-salinity site ($p = 0.01$) and the oiled low-salinity site ($p = 0.037$). There were no significant differences between the control and oiled high-salinity sites ($p = 0.087$).

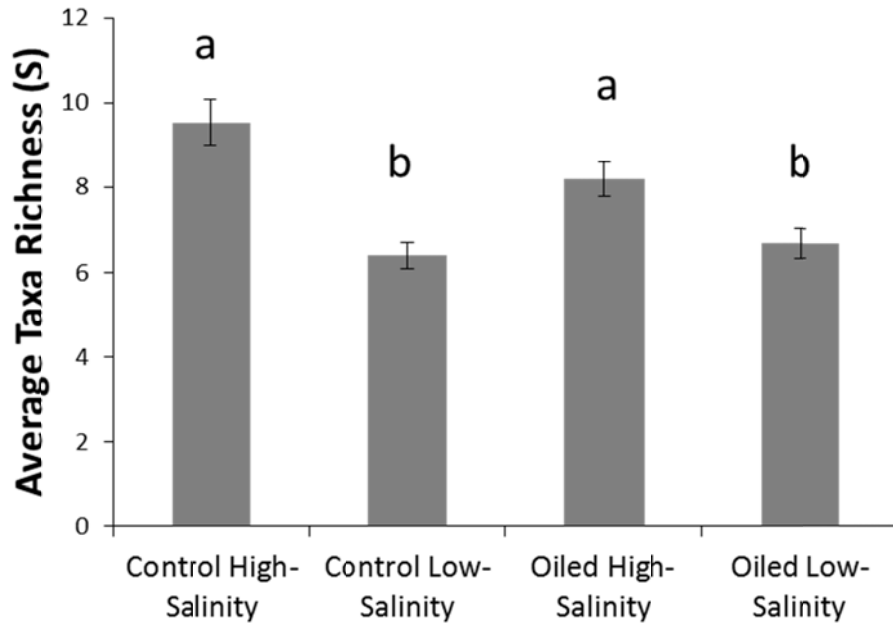


Figure 5. Mean taxa richness (S) values \pm standard errors between four field sites, pooled over three sampling months in 2013. Letters above histograms indicate significant differences in taxa richness from Tukey's *a posteriori* tests.

In 2012, only season had a significant effect on the Shannon-Weiner diversity index (Table 4, Figure 6). However, the season by site interaction was also significant (Table 4). When comparing months within field sites, there were only significant differences between May and August ($p < 0.001$), and between August and November ($p < 0.001$) at the oiled low-salinity site. There were no significant differences between months at the control high-salinity, control low-salinity, or oiled high-salinity sites. There were no significant differences between sites in May 2012. In August, there were significant differences between the control high-salinity and oiled low-salinity sites ($p = 0.003$), the control low-salinity and oiled low-salinity sites ($p = 0.018$), and between the oiled high-salinity and oiled low-salinity sites ($p < 0.001$). In November, there was only a significant difference between the control low-salinity and oiled low-salinity sites ($p = 0.001$).

Both month and field site had significant effects (Table 4, Figure 6) on commensal diversity in 2013. There was also a significant month by site interaction (Table 4). Comparing months within sites indicated a significant difference between August and November at the control high-salinity site ($p = 0.002$), but not between May and August or between May and November. At the oiled high-salinity site there were significant differences between May and August ($p = 0.015$) and between May and November ($p < 0.001$), but not between August and November. There were no significant differences between months at the control low-salinity or oiled low-salinity sites. When comparing the field sites between sampling months, there were significant differences between the control high-salinity and oiled high-salinity sites ($p < 0.001$), the control high-salinity and the oiled low-salinity sites ($p = 0.001$), the control low-salinity and oiled high-salinity sites ($p = 0.002$), and the control low-salinity and oiled low-salinity sites ($p = 0.029$) in May 2013. There were no significant differences between sites in August 2013. In November 2013 there were only significant differences between the control high-salinity and control low-salinity sites ($p = 0.001$) and the control high-salinity and oiled low-salinity sites ($p = 0.002$).

Table 4. F and P values (in parentheses) of the main effects and interaction terms for each variable (total commensal abundance, taxa richness, and Shannon-Weiner diversity) for both 2012 and 2013.

	2012			2013		
	Abundance	Richness	Diversity	Abundance	Richness	Diversity
Month	4.79 (0.005)	0.96 (0.389)	6.82 (0.003)	39.31 (<0.001)	0.42 (0.657)	9.93 (0.002)
Site	4.87 (0.012)	1.58 (0.206)	1.88 (0.145)	15.87 (<0.001)	13.11 (<0.001)	15.44 (0.001)
Month x Site	4.43 (0.001)	5.36 (0.003)	12.5 (<0.001)	18.66 (<0.001)	1.96 (0.09)	9.57 (<0.001)
Error Degrees of Freedom	48	48	48	48	48	48

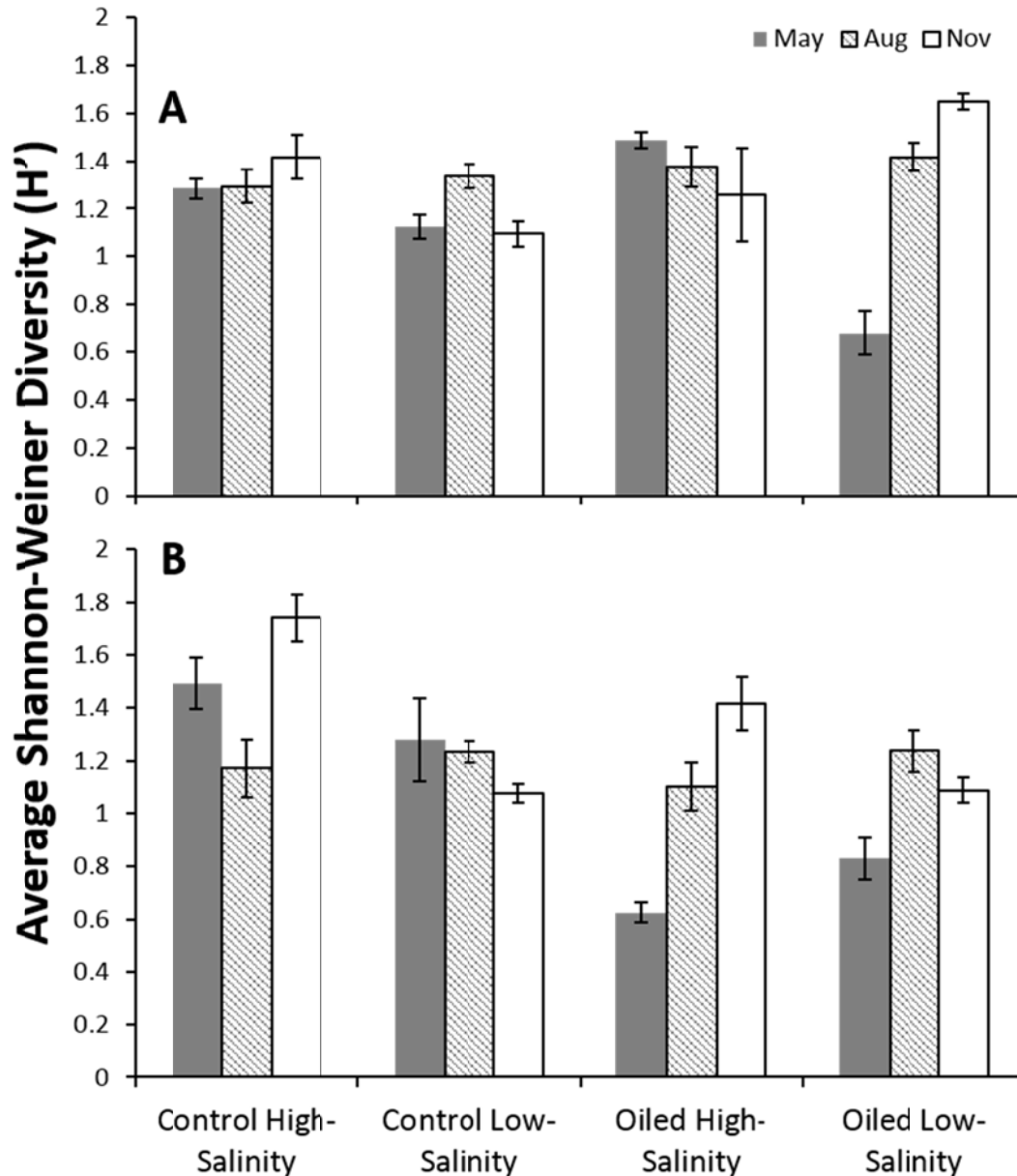


Figure 6. Mean Shannon-Weiner Diversity values \pm standard errors for at four field sites across three sampling months during (A) 2012 and (B) 2013

Acute Oil Exposure Experiment

In June 2013, there were significant differences among colonization periods (Table 5) on commensal abundance, but no treatment effect. The week by treatment interaction was not significant. Tukey's *a posteriori* comparison of the main effects (Figure 7) indicated Week 1 was significantly different from Week 2 ($p = 0.015$), Week 4 ($p = 0.003$), and Week 8 (p

<0.001). Week 2 was not significantly different from Week 4 ($p = 0.948$) but was significantly different from Week 8 ($p < 0.001$), and Week 4 was significantly different from Week 8 ($p = 0.001$).

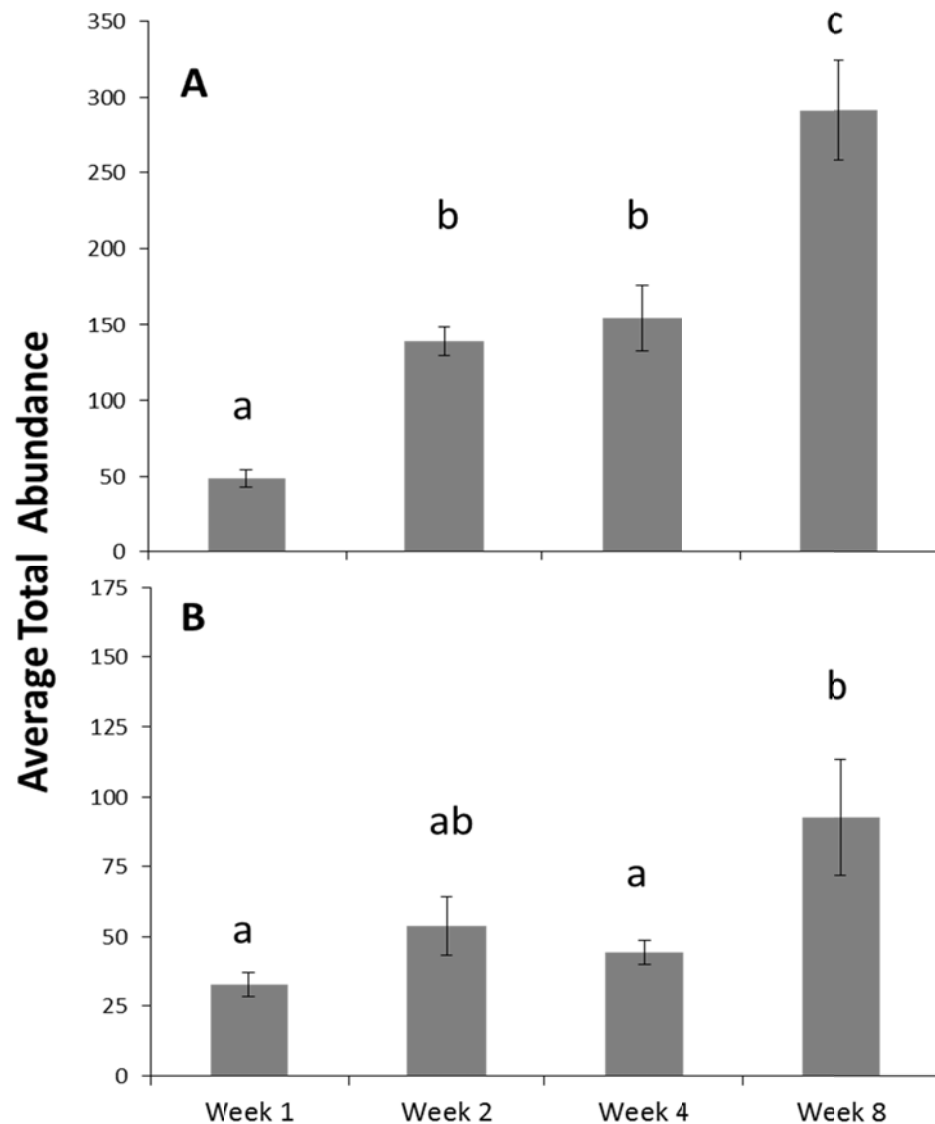


Figure 7. Mean total commensal abundance \pm standard errors for four colonization periods in (A) June and (B) September 2013, pooled over both treatments. Letters above histograms indicate significant differences in total abundance from Tukey's *a posteriori* tests.

When the experiment was replicated in September 2013, the log of total commensal abundance was significantly different for both main effects, but there was no significant interaction (Table 5). Based on Tukey's *a posteriori* comparison of the main effects (Figure 7),

Week 1 was significantly lower than Week 8 ($p = 0.001$); however, there was no significant difference between Week 1 and Weeks 2 and 4 ($p = 0.487$ and 0.362 , respectively). There was no significant difference in commensal abundance between Week 2 and Week 4 ($p = 0.996$), between Week 2 and Week 8 ($p = 0.055$), or between Week 4 and Week 8 ($p = 0.09$). Total commensal abundance was significantly lower for the oil treatment than for the control ($p < 0.001$) (Figure 8).

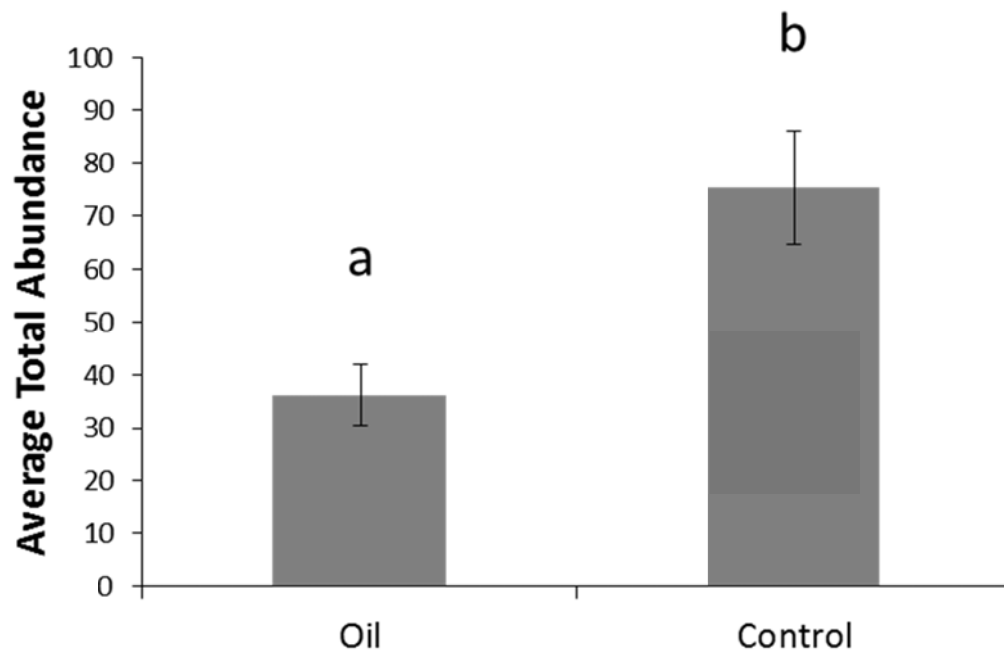


Figure 8. Mean total commensal abundance per bag \pm standard errors between oil-soaked and control treatments in September 2013, pooled over time. Letters above histograms indicate significant differences in total abundance from Tukey's *a posteriori* tests.

In June 2013, there was no significant treatment effect on the log of taxa richness, but colonization time had a significant effect and the time by treatment interaction term significant (Table 5). Tukey's *a posteriori* comparison of the main effects (Figure 9) showed Week 8 taxa richness was significantly higher than Week 1 ($p = 0.003$), Week 2 ($p = 0.011$), and Week 4 ($p = 0.017$), but no other significant differences between weeks existed.

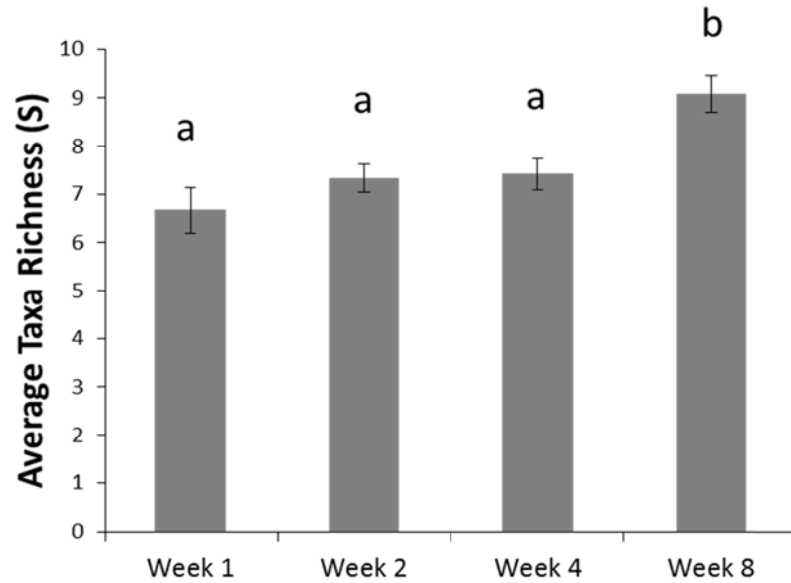


Figure 9. Mean taxa richness (S) values \pm standard errors on four different sampling time periods in June 2013, pooled over both treatments. Letters above histograms indicate significant differences in taxa richness from Tukey's *a posteriori* tests.

In September 2013, there was a significant treatment effect on taxa richness (Table 5).

There was no significant difference between weeks, nor was there a significant week by treatment interaction (Table 5). Tukey's *a posteriori* test of the main effects revealed a significantly lower taxa richness for the oil treatment than for the control ($p = 0.002$) (Figure 10).

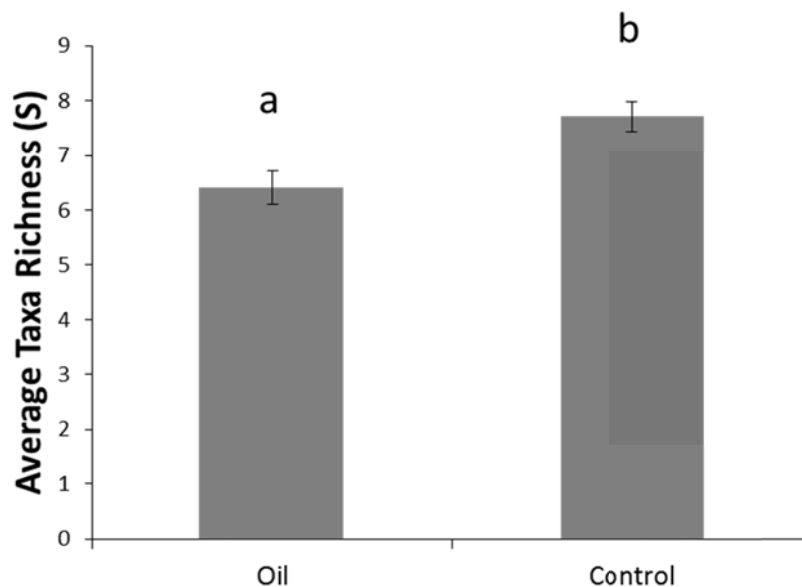


Figure 10. Mean taxa richness (S) values \pm standard errors between oil-soaked and control treatments in September 2013, pooled over time. Letters above histograms indicate significant differences in taxa richness from Tukey's *a posteriori* tests.

In June 2013, there was a significant colonization period effect on Shannon-Weiner commensal diversity but not a significant treatment effect (Table 5). The week by treatment interaction was also significant (Table 5, Figure 11). Comparing treatments within weeks indicated no significant differences. When comparing weeks within the oil treatment, Week 1 was significantly different than Week 4 ($p < 0.001$) and Week 8 ($p < 0.001$), and Week 2 was significantly different than Week 4 ($p = 0.01$) and Week 8 ($p = 0.01$). When comparing weeks between the control treatment, Week 4 was significantly different from Week 1 ($p < 0.001$), Week 2 ($p = 0.033$), and Week 8 ($p = 0.043$).

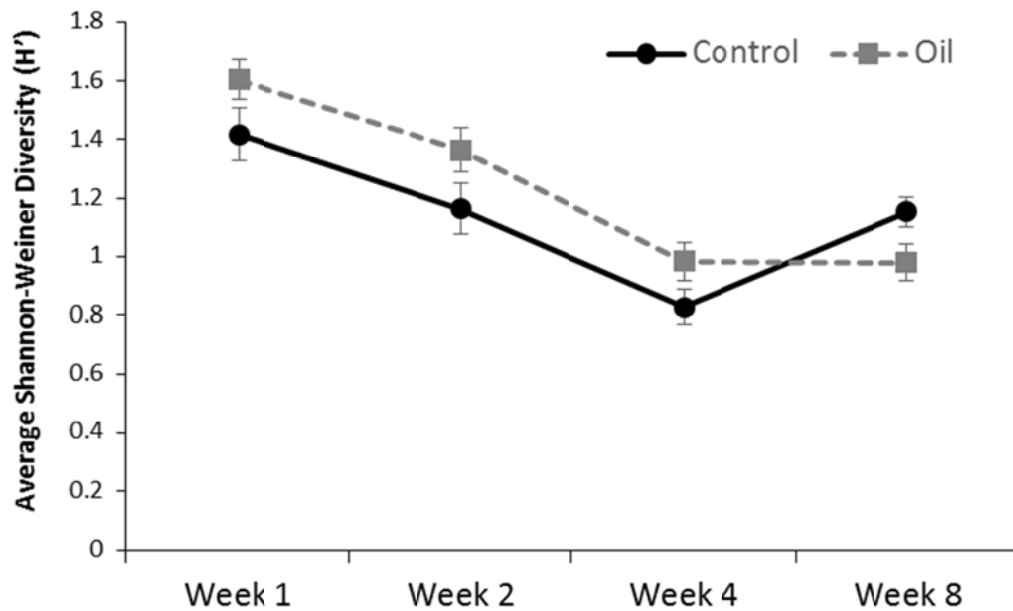


Figure 11. Mean Shannon-Weiner Diversity (H') values \pm standard errors for oil-soaked and unsoiled control shell over four different sampling intervals in June 2013.

In September 2013, there was a significant colonization period effect on Shannon-Weiner diversity but no significant treatment effect or week by treatment interaction (Table 5). Tukey's *a posteriori* test indicated that taxa diversity was significantly reduced in Week 8 compared to Week 1 ($p = 0.022$), Week 2 ($p = 0.001$), and Week 4 ($p = 0.01$); however, no other significant differences between weeks existed (Figure 12).

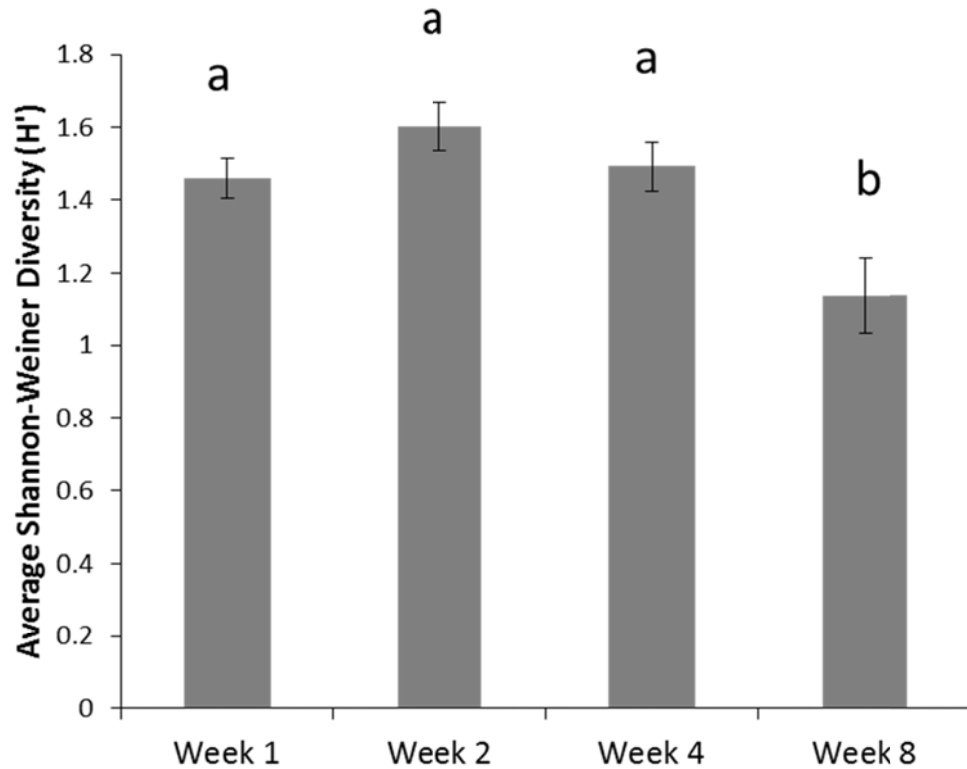


Figure 12. Mean Shannon-Weiner Diversity (H') values \pm standard errors on four different sampling time periods in September 2013, pooled over both treatments. Letters above histograms indicate significant differences in diversity from Tukey's *a posteriori* tests.

In both June and September 2013, commensal abundance significantly increased with increasing colonization time, yet the Shannon-Weiner diversity index generally decreased with time. It was theorized that this trend was the result of a few taxa groups dominating the commensal community composition by Week 8. Stacked histograms were created to depict the differences in relative abundance of the commensal taxa over time for each treatment (Figure 13). The figures indicate that class Polychaeta was in fact dominant over all other taxa groups by Week 8 in both treatments in both June and September 2013.

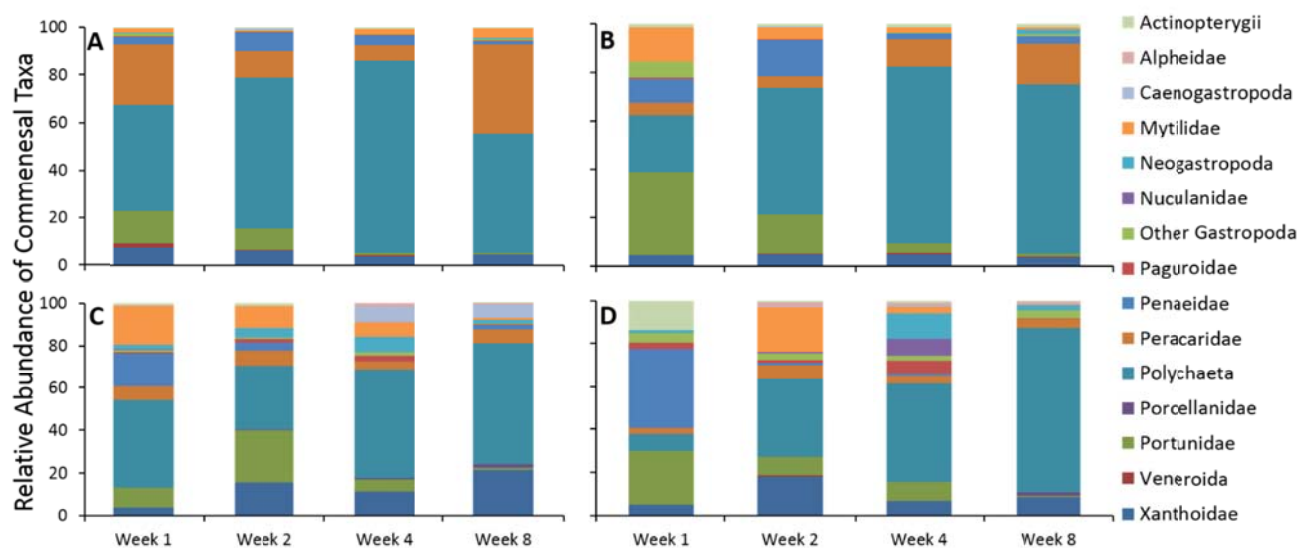


Figure 13. Relative abundance of commensal taxa groups over time for both June 2013 (A) control and (B) oil treatments and September 2013 (C) control and (D) oil treatments. Each colored section represents a different taxon (See Appendix for common names).

Table 5. F and P values (in parentheses) of the main effects and interaction terms for each variable (total commensal abundance, taxa richness, and Shannon-Weiner diversity) for both June and September.

	June			September		
	Abundance	Richness	Diversity	Abundance	Richness	Diversity
Week	24.93 (<0.001)	7.68 (0.004)	26.97 (<0.001)	5.7 (0.002)	1.13 (0.347)	7.11 (0.001)
Treatment	3.21 (0.081)	1.2 (0.279)	3.56 (0.066)	29.97 (<0.001)	10.38 (0.003)	2.66 (0.11)
Week x Treatment	0.8 (0.499)	0.46 (0.715)	3.11 (0.037)	2.37 (0.085)	1.57 (0.213)	0.83 (0.483)
Error Degrees of Freedom	40	40	40	40	40	40

In June 2013, there were significant colonization interval effects ($p < 0.001$) and treatment effects ($p = 0.021$) on the number of live spat counted on experimental cultch; however, there was no week by site interaction ($p = 0.064$). Tukey's *a posteriori* test of the main effects indicated that Week 1 had significantly lower spat abundance than Week 2 ($p = 0.035$), Week 4 ($p < 0.001$), and Week 8 ($p = 0.001$) (Figure 14). Week 2 had significantly lower spat abundance than Week 4 ($p = 0.018$) but was not significantly different from Week 8 ($p = 0.638$). There was

no significant difference in spat abundance from Week 4 to Week 8 ($p = 0.236$). There was also a significant difference in live spat abundance between the oil-soaked and unoiled control treatments ($p = 0.021$) (Figure 15).

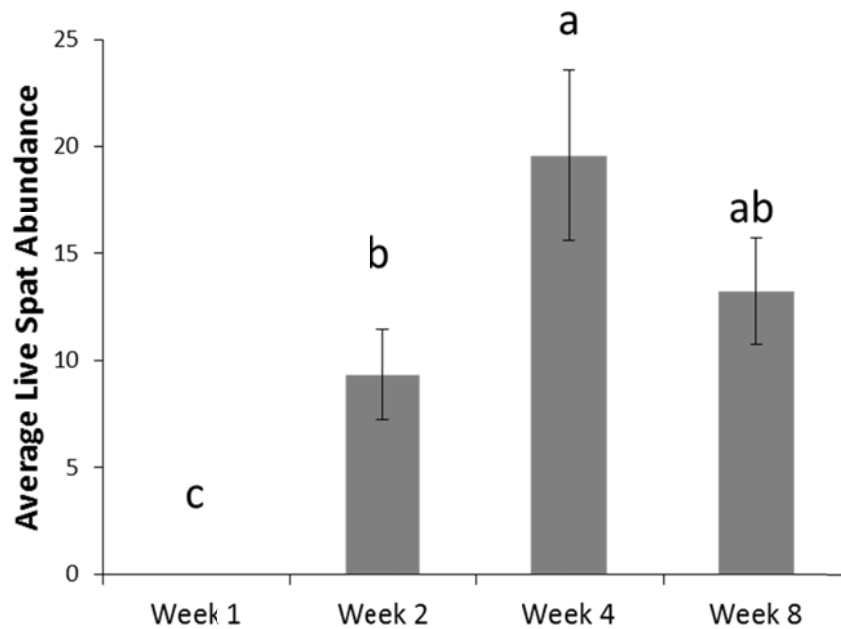


Figure 14. Mean live spat abundance per bag \pm standard errors on four different colonization periods in June 2013, pooled over both treatments. Letters above histograms indicate significant differences in spat abundance from Tukey's *a posteriori* tests.

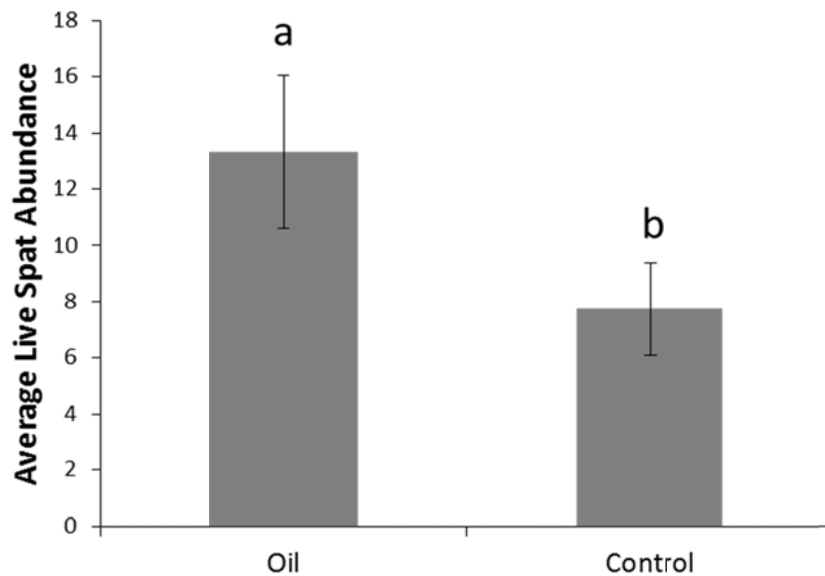


Figure 15. Mean live spat abundance per bag \pm standard errors between oil-soaked and control treatments in June 2013, pooled over time. Letters above histograms indicate significant differences in spat abundance from Tukey's *a posteriori* tests.

When the experiment was replicated in September 2013, there was a significant difference between weeks ($p = 0.001$), as well as between treatments ($p = 0.049$), on live spat abundance. The week by treatment interaction was also significant ($p = 0.038$) (Figure 16), driven largely by Week 8, where the oiled sites had higher recruitment compared to control sites. Comparing treatments within weeks indicated no significant differences. When comparing weeks within the oil treatment, Week 8 was significant different from Week 1 ($p < 0.001$), Week 2 ($p = 0.001$), and Week 4 ($p = 0.029$). When comparing weeks between the control and oil treatment, Week 8 was significant different from Week 1 ($p = 0.027$) and Week 2 ($p = 0.047$). There were no other significant differences between weeks for either treatment.

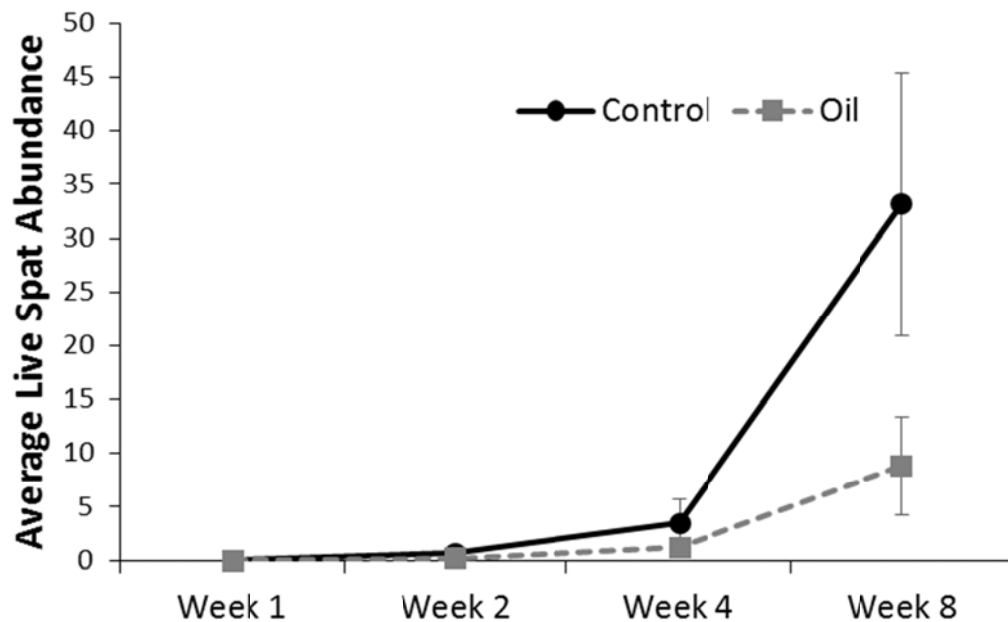


Figure 16. Mean live spat abundance per bag \pm standard errors for oil-soaked and unoiled control shell over four different sampling intervals in September 2013.

The spat recruitment data was also measured for spat length. In June 2013 there was a significant colonization period effect ($p < 0.001$) but no treatment effect ($p = 0.026$) on spat growth. The week by treatment interaction was not significant ($p = 0.604$). Tukey's *a posteriori* test (Figure 17) indicated that all four weeks were significantly different for each other; spat

length was significantly lower in Week 1 than Week 2 ($p = 0.003$), Week 4 ($p < 0.001$), and Week 8 ($p < 0.001$). Week 2 was significantly lower than Week 4 ($p < 0.001$) and Week 8 ($p < 0.001$). Week 4 was significantly lower than Week 8 ($p < 0.001$). In September 2013, there was a significant colonization period effect ($p = 0.001$) on spat length, but no treatment effect ($p = 0.152$) and no week by treatment interaction ($p = 0.265$). Tukey's *a posteriori* test (Figure 17) indicated that spat length was significantly greater in Week 8 than Week 1 ($p = 0.001$) and Week 2 ($p = 0.002$).

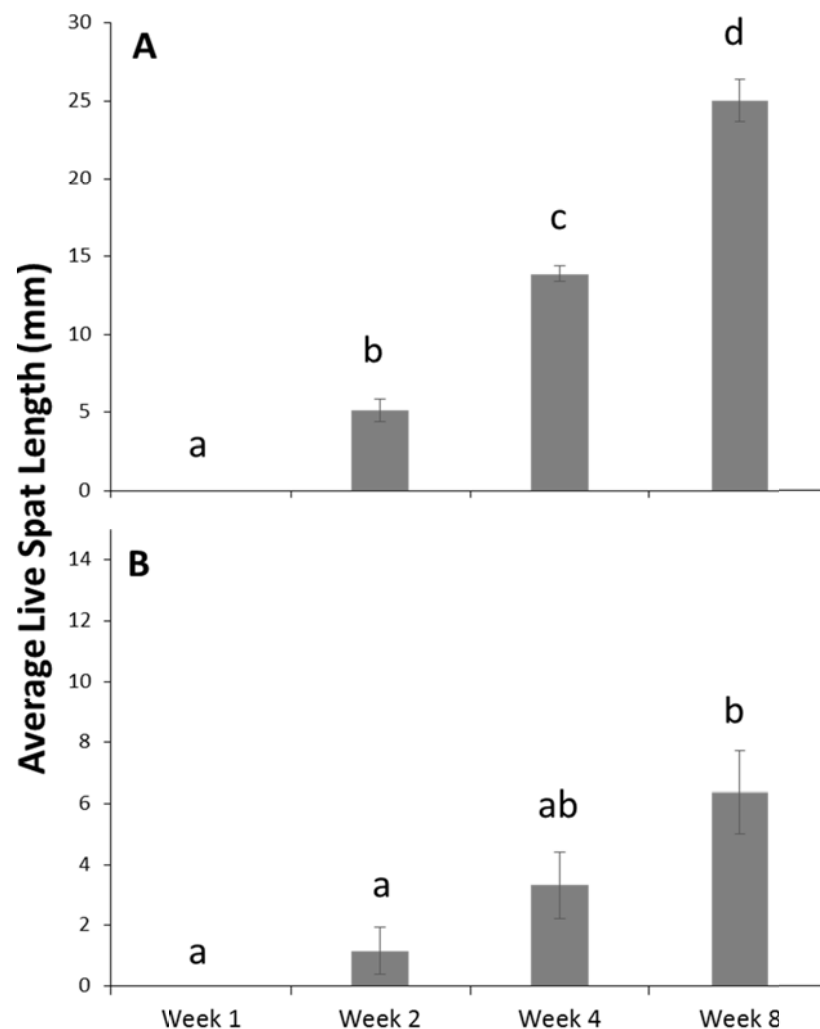


Figure 17. Mean live spat length \pm standard errors on four different sampling time periods in (A) June and (B) September 2013, pooled over both treatments. Letters above histograms indicate significant differences in diversity from Tukey's *a posteriori* tests.

Artificial Reef Substrate Material Experiment

When the commensal bags containing different artificial reef construction materials were analyzed for the log of total organismal abundance in June 2013, there were significant differences between the main treatment effects (Table 6), but there was no site by substrate interaction (Table 6). Using Tukey's *a posteriori* tests to compare differences among sites (Figure 18), the control high-salinity site was significantly different from all other sites; the control high-salinity site was higher in commensal abundance than the control low-salinity site ($p < 0.001$), the oiled high-salinity site ($p < 0.001$), and the oiled low-salinity site ($p < 0.001$). The control low-salinity was significantly lower in abundance than the oiled high-salinity site ($p = 0.007$) but was not significantly different from the oiled low-salinity site ($p = 0.325$). There was no significant difference between the oiled high-salinity and the oiled low-salinity site ($p = 0.359$). Tukey's *a posteriori* tests (Figure 19) indicated no significant differences between OysterCrete and Shell ($p = 0.32$), but that abundance in Rock was significantly lower than both OysterCrete and Shell.

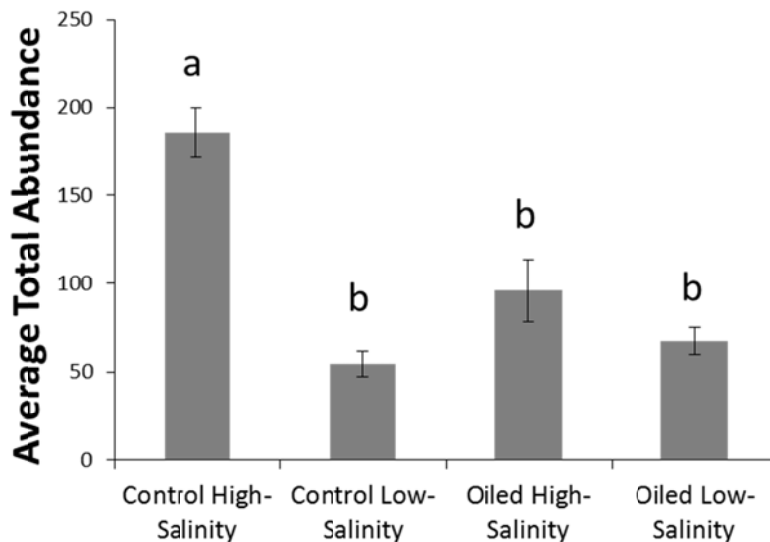


Figure 18. Mean total commensal abundance per bag \pm standard errors among four field sites in June 2013, pooled across all three substrate types. Letters above histograms indicate significant differences in total abundance from Tukey's *a posteriori* tests.

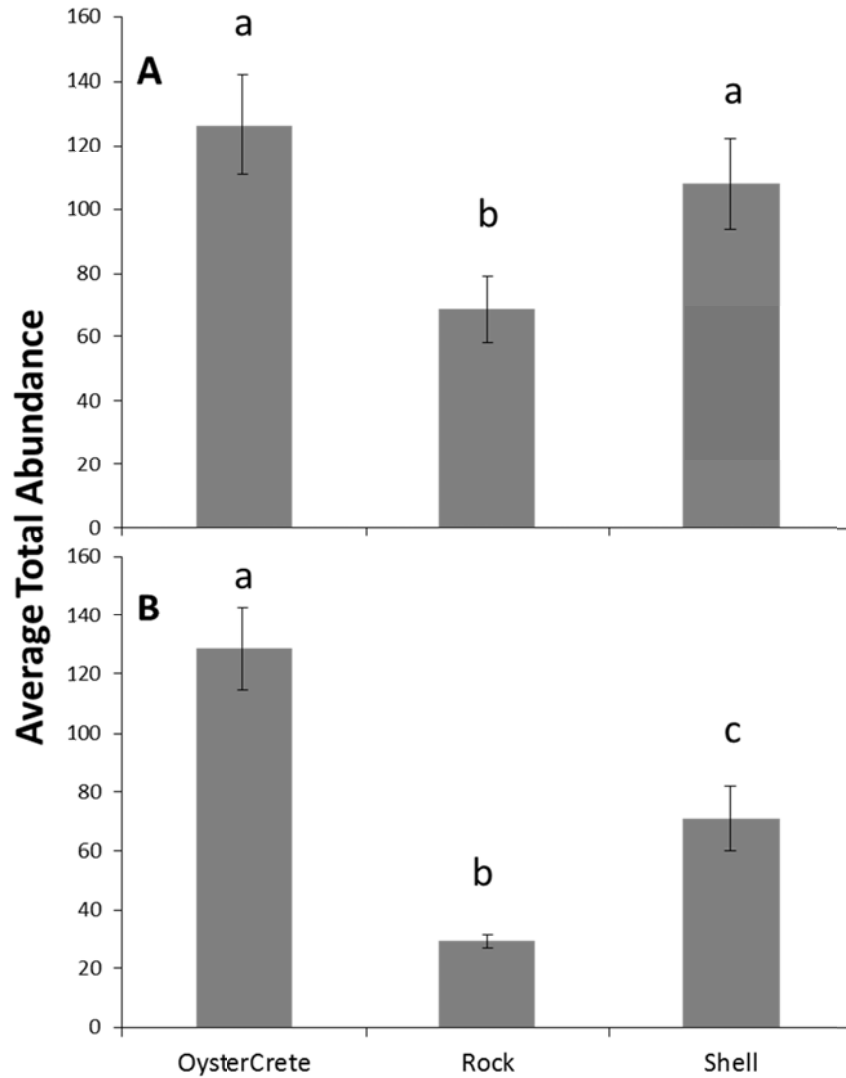


Figure 19. Mean total commensal abundance per bag \pm standard errors between three artificial oyster reef substrate materials in (A) June and (B) September 2013, pooled across all four sites. Letters above histograms indicate significant differences in total abundance from Tukey's *a posteriori* tests.

When the experiment was replicated in September 2013, only the substrate type had a significant effect on the log of total commensal abundance (Table 6). There was also no significant interaction between sites and substrate types (Table 6). Tukey's *a posteriori* test indicated that all three substrate treatments were significantly different from each other (Figure 19); OysterCrete was significantly higher in commensal abundance than both Rock ($p < 0.001$) and Shell ($p = 0.005$), and Shell was significantly greater than Rock ($p = 0.001$).

In June 2013, both the sampling site and substrate type had a significant effect on taxa richness (Table 6). There was no significant site by substrate interaction (Table 6). Using Tukey's *a posteriori* test to compare the main substrate effects (Figure 20) indicated that Rock and Shell were significantly different ($p = 0.041$), but there were no significant differences between OysterCrete and Rock ($p = 0.23$) or between OysterCrete and Shell ($p = 0.69$). When comparing main site effects (Figure 21) indicated that the control high-salinity site had significantly higher taxa richness than the control low-salinity site ($p = 0.04$) and the oiled low-salinity site ($p < 0.001$), but was not significantly different from the oiled high-salinity site ($p = 0.845$). The control low-salinity site was significantly higher in taxa richness than the oiled low-salinity site ($p = 0.013$) but was not significantly different from the oiled high-salinity site ($p = 0.233$). The oiled high-salinity was significantly higher in taxa richness than the oiled low-salinity site ($p < 0.001$).

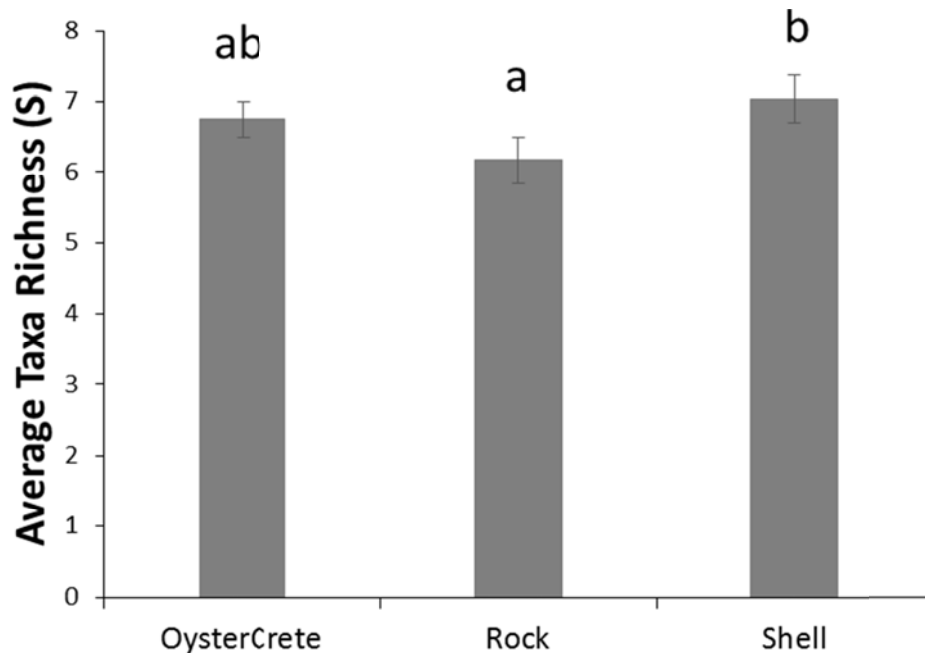


Figure 21. Mean taxa richness (S) values \pm standard errors between three artificial oyster reef substrate materials in June 2013, pooled across all sites. Letters above histograms indicate significant differences in taxa richness from Tukey's *a posteriori* tests.

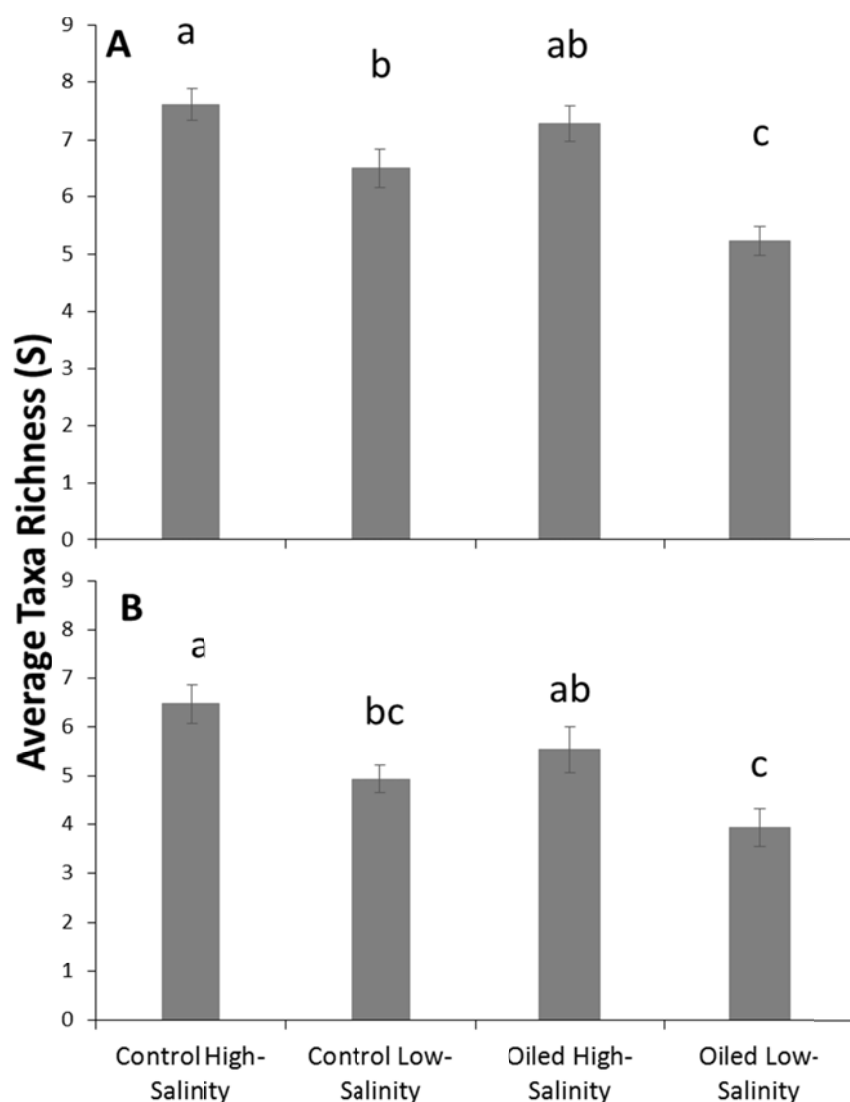


Figure 21. Mean taxa richness (S) values \pm standard errors between four field sites in (A) June and (B) September 2013, pooled across all three substrate types. Letters above histograms indicate significant differences in taxa richness from Tukey's *a posteriori* tests.

In September 2013, there was a significant difference in taxa richness among sites but not substrate types (Table 6). There was no site by substrate interaction (Table 6). Tukey's *a posteriori* test of main site effects (Figure 21) indicated that the control high-salinity site was significantly greater in taxa richness than the control low-salinity site ($p = 0.039$) and the OLS site ($p = 0.002$), but was not significantly different from the control high-salinity site ($p = 0.344$). The control low-salinity site was not significantly different from the oiled high-salinity site ($p =$

0.63) or the oiled low-salinity site ($p = 0.346$). The oiled high-salinity was significantly greater in taxa richness than the oiled low-salinity site ($p = 0.029$).

In June 2013, there was a significant site effect on commensal diversity, but no significant substrate effect or site by substrate interaction (Table 6). Using Tukey's *a posteriori* test (Figure 22), the control high-salinity site was significantly different from the control low-salinity site ($p = 0.007$) and the oiled low-salinity site ($p = 0.015$), but not the oiled high-salinity site ($p = 0.222$). The control low-salinity site was significantly greater in diversity than the oiled low-salinity site ($p < 0.001$) but was not significantly different from the oiled high-salinity site ($p = 0.493$). The oiled high-salinity was significantly higher in diversity than the oiled low-salinity site ($p < 0.001$).

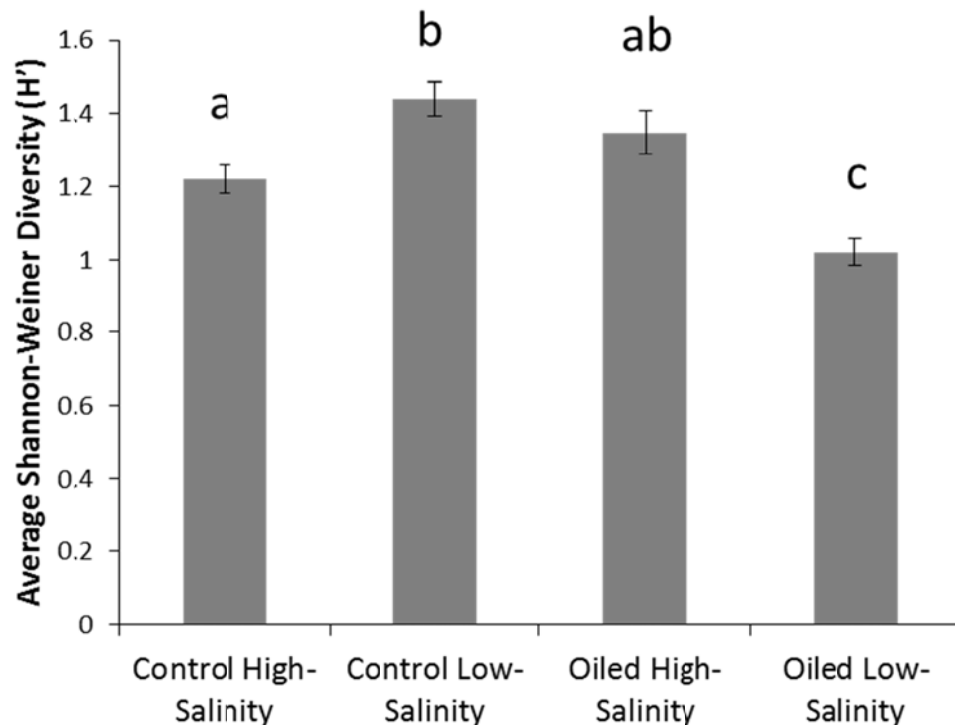


Figure 22. Mean Shannon-Weiner Diversity (H') values \pm standard errors between four field sites in June 2013, pooled across all three substrate types. Letters above histograms indicate significant differences in diversity from Tukey's *a posteriori* tests.

In September 2013, both the main effects were significant, and there was a significant site by substrate interaction (Table 6, Figure 23). When examining substrate types among sites, the only significant difference occurred between shell and rock at the control high-salinity site ($p = 0.0156$). When comparing sites among substrate materials there were no significant differences between sites for the oyster shell substrate, and for the OysterCrete treatment there was only a significant difference between the control high-salinity and oiled low-salinity sites ($p = 0.041$). The limestone rock substrate was significantly different between the control high-salinity and control low-salinity sites ($p = 0.002$) and between the control high-salinity and oiled low-salinity sites ($p = 0.004$).

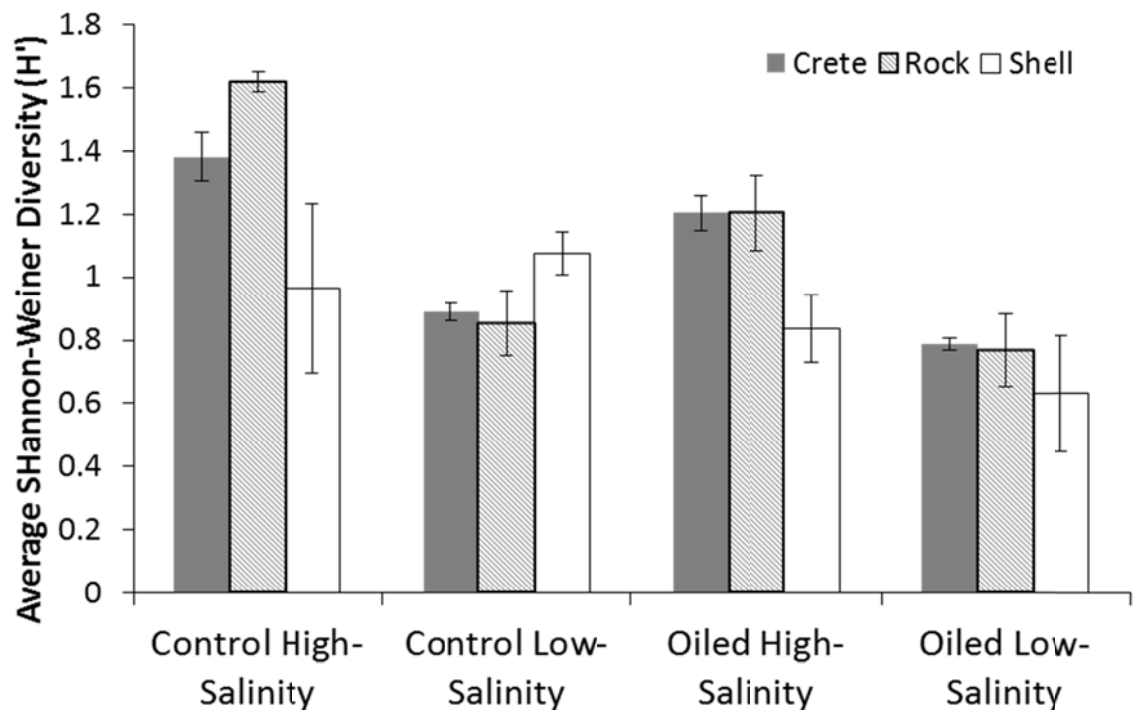


Figure 23. Mean of Shannon-Weiner Diversity (H') values \pm standard three artificial oyster reef substrate materials at four field sites in September 2013.

Table 6. F and P values (in parentheses) of the main effects and interaction terms for each variable (total commensal abundance, taxa richness, and Shannon-Weiner diversity) for both June and September.

	June			September		
	Abundance	Richness	Diversity	Abundance	Richness	Diversity
Site	29.65 (<0.001)	13.64 (<0.001)	15.67 (<0.001)	1.98 (0.13)	7.34 (0.004)	13.01 (<0.001)
Substrate	14.4 (<0.001)	3.21 (0.048)	0.21 (0.811)	25.73 (<0.001)	1.52 (0.228)	4.32 (0.019)
Site x Substrate	1.32 (0.261)	0.76 (0.602)	1.3 (0.273)	1.03 (0.418)	0.69 (0.662)	2.66 (0.026)
Error Degrees of Freedom	60	60	60	48	48	48

In June 2013, there were significant site ($p < 0.001$) and substrate ($p = 0.002$) effects on the abundance of live spat collected, and the site by substrate interaction was also significant ($p < 0.001$) (Figure 24). When comparing substrate types within sites, the only significant differences occurred between OysterCrete and Shell ($p < 0.001$) and between Rock and Shell ($p < 0.001$) at the oiled high-salinity site. When comparing sites within substrate types, the oiled high-salinity site was significantly different from the control high-salinity site ($p < 0.001$), the control low-salinity site ($p < 0.001$), and the oiled low-salinity site ($p < 0.001$) for the Shell treatment. No other significant site differences among substrate types existed.

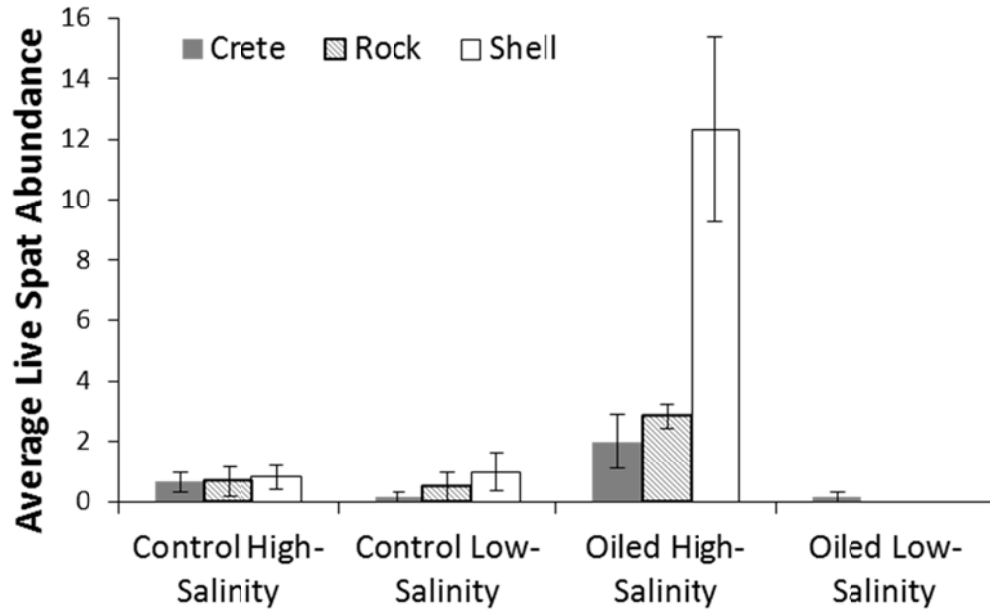


Figure 24. Mean live spat abundance per bag \pm standard errors between three substrate types at four field sites in June 2013.

When the experiment was replicated in September 2013, there was a significant site effect ($p < 0.001$) on live spat abundance, but the substrate effect was not significant ($p = 0.154$). The site by substrate interaction was also not significant ($p = 0.087$). Using Tukey's *a posteriori* test of the main effects (Figure 25), the control low-salinity site was significantly higher in spat abundance than the control high-salinity site ($p < 0.001$), the oiled high-salinity site ($p < 0.001$), and the oiled low-salinity site ($p < 0.001$). There were no significant differences in spat abundance between the control high-salinity site and the oiled high-salinity site ($p = 0.114$) or the oiled low-salinity site ($p = 0.558$), or between the oiled high-salinity site and the oiled low-salinity site ($p = 0.763$).

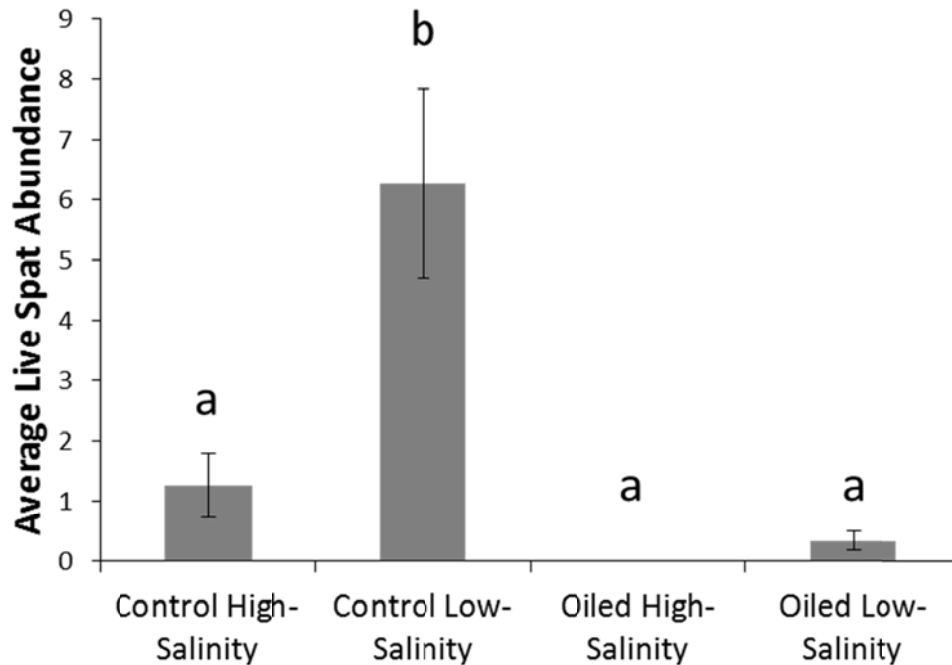


Figure 25. Mean live spat abundance per bag \pm standard errors at four field site in September 2013, pooled across three substrate types. Letters above histograms indicate significant differences in diversity from Tukey's *a posteriori* tests.

The data for spat recruitment were also measured for spat length. In June 2013, there was a significant site effect on spat length ($p < 0.001$), but no significant substrate effect ($p = 0.15$) or site by substrate interaction ($p = 0.491$). Tukey's *a posteriori* test (Figure 26) indicated that the oiled high-salinity site was significantly greater in spat length than the oiled low-salinity site ($p < 0.001$), the control high-salinity site ($p = 0.035$), and the control low-salinity site ($p = 0.001$). The control high-salinity site was significantly greater in spat length than the oiled low-salinity site ($p = 0.007$), but there was no difference in spat length between the control low-salinity site and either the oiled low-salinity or control high-salinity sites. In September 2013, there was also a significant site effect on spat length ($p < 0.001$), but no substrate effect ($p = 0.994$) or site by substrate interaction ($p = 0.197$). Tukey's *a posteriori* test (Figure 26) indicated that the control low-salinity site had significantly higher spat growth rates than the control high-salinity site (p

<0.001), the oiled high-salinity site ($p < 0.001$), and the oiled low-salinity site ($p = 0.002$). There were no other significant differences in spat length between sites.

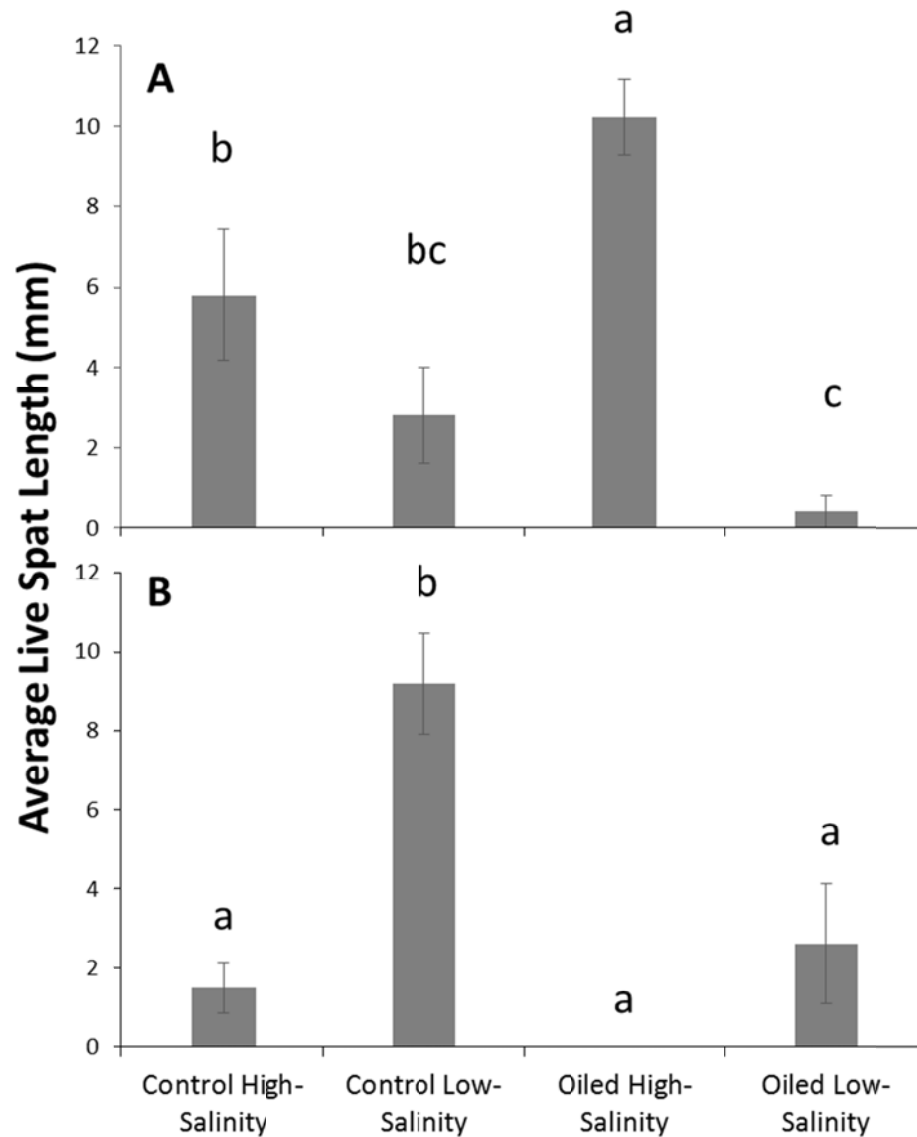


Figure 26. Mean live spat length \pm standard errors at four different field site in (A) June and (B) September 2013, pooled over three substrate treatments. Letters above histograms indicate significant differences in diversity from Tukey's *a posteriori* tests.

DISCUSSION

In general, salinity and seasonal variation were more important for explaining differences in commensal community than the presence of hydrocarbons. When oil was an important factor, based on significant differences between oil-contaminated and control sites, commensal abundance was surprisingly greater at oiled sites in every sampling month for both 2012 and 2013. Previous studies have found that the presence of hydrocarbons negatively affected the abundance and diversity of oyster reef commensal assemblages (Hulathduwa and Brown 2006), yet the results of this study indicate that hydrocarbon contamination may possibly enhance commensal abundance in the years following an oil spill. Further examination of the post hoc comparisons, however, indicated that out of the 132 pairwise contrasts, an oil effect only accounted for less than one-third of significant differences in both commensal abundance and diversity. The remaining significant differences were the result of other factors such as differences in salinity, seasonal variation, or an oil-salinity interaction. Ultimately, the long-term effects of hydrocarbon contamination on commensal abundance and diversity, while statistically significant, were neither large or nor consistent, and most likely do not have significant biological implications.

Wells (1961) reported that salinity is the most important factor determining the distribution of oyster commensal communities. The results for taxa richness corroborate Wells' findings in that the number of taxa groups was consistently greater at the high salinity sites than at low salinity sites. Furthermore, 25% of the post hoc differences in taxa richness were attributed to salinity effects and 50% were attributed to the season-salinity interaction in 2012. There was no

effect from hydrocarbon contamination alone on the number of commensal organism taxa groups. While statistically significant, the differences in mean taxa richness between high and low salinity sites or between oiled and control sites were only that of a few taxa groups, which again may not be biologically significant.

In 2012, snails in the family Caenogastropoda were present at oiled sites but not control sites, and the brittle star (order Ophiuroidea) was only collected at control sites, yet the relative abundance was so low that it would be considered an outlier as opposed to having any true biological significance. In 2013, there were no nemerteans flat worms (order Hoplonemertea) or sea squirts (family Styelidae) collected at the low salinity sites; however, these taxa only represented a small proportion of the commensal communities found at the high salinity sites, so their presence may not be biologically significant. At all sites, worms in class Polychaetes were the dominant taxa.

Data from 2012 sediment samples collected at the four field sites revealed that while the oiled low-salinity site did have the highest concentration of tPAH (ng/g), even the unoiled control sites produced relatively high levels of tPAH (ng/g). In areas such as the northern Gulf of Mexico, where spills and leaks from oil production operations are fairly common, local fauna may be pre-adapted to hydrocarbon exposure (McCoy and Brown 1998; Carman et al. 2000). This could provide one possible explanation for the lack of a greater oil effect on the commensal community structure, especially via uptake from the surrounding environment.

Increased temperatures are known to enhance the rate of oil degradation (Atlas 1991), both by changing the physical and chemical structure of the hydrocarbons, as well as accelerating the rates of hydrocarbon metabolism by microorganisms (Ortman et al. 2012). A study by McCoy and Brown (1998) found that after six weeks any harmful effects produced by oil-contaminated

substrates were diminished due to weathering in the field. Despite the tPAH (ng/g) levels found in the sediment samples in Barataria Bay, perhaps the Maconda-252 well oil has degraded to a level at which it no longer produces a deleterious response on the oyster reef commensal assemblages.

The general trend of the long-term monitoring of oyster commensal community response to *Deepwater Horizon* thus indicates that the oil effect appears to diminish over time. In the short-term oil contamination experiment, even though abundance significantly increased with time, it is important to note that oiling did not prevent immediate colonization, as organisms were collected one week after the deployment of oil-soaked substrates in both replicates. It is also noteworthy that colonization continued throughout the 8-week experiment, and mean commensal abundance was nearly identical between treatments after 8 weeks, indicating that after 8 weeks any oil effects were lost, perhaps due to weathering or biodegradation. In this study, no analysis of the remaining hydrocarbons was conducted following each immersion time interval, which should be a consideration for future studies to better understand the lingering chemical properties on heavily contaminated substrates.

In the September replicate, the unoiled cultch was significantly greater in commensal abundance compared to the oiled substrate. The number of organisms collected was much greater in June than September, however, which could indicate that seasonal variation may affect the rate of oil degradation on contaminated substrates. Furthermore, the 8-week collection of this replicate occurred during the last week of October 2013; reduced temperatures could also affect the overall abundance and distribution of certain species.

The trends in taxa richness were inconsistent with respect to time and oil treatments. Furthermore, the variation in the number of taxa represented was a difference of only one or two

taxa groups, indicating no major oil effect even when a statistically significant difference existed between control and oil-soaked cultch. There was also no significant oil effect on the diversity of commensal taxa, although diversity significantly decreased over time. After 8 weeks the commensal bags were dominated by only a few taxa groups, primarily class Polychaeta with the family Mytilidae being the second most abundant group, as overall abundance increased, accounting for the decrease in diversity. These results agree with the findings of Peterson (2001), who found an increase in abundance of deposit-feeding benthic infauna following the Exxon Valdez oil spill, citing that this increase in abundance could likely be attributed to either an increase in hydrocarbon-degrading bacteria within the sediment or a reduction of more oil-sensitive predators. These hypotheses may provide explanation of the community being mostly comprised of polychate worms after 8 weeks; however, the number of taxonomic groups also varied by season, inferring that seasonal variation or abiotic factors may play a greater role than hydrocarbon contamination on commensal distribution.

The recruitment of oyster spat was also significantly affected by both colonization time as well as an oil effect. In June 2013, the abundance of spat significantly increased over time through Week 4, but decreased at Week 8, perhaps indicating that predation started to have an effect on spat survival. When the experiment was replicated in September 2013, spat abundance significantly increased over time without experiencing the same decline between Weeks 4 and 8. This corroborates with the decreased commensal abundance also reported in these results, and also verifies that the absence of more predatory commensal organisms enhances spat recruitment. By Weeks 4 and 8 the class Polychaeta was the dominant taxon, which is generally not an important predator of oyster spat. Conversely, the blue crab *Callinectes sapidus* (family Portunidae), mud crabs in the superfamily Xanthoidea, and the Gulf oyster drill *Stramonita*

haemastoma (order Neogastropoda) are all voracious predators of juvenile oysters (Menzel and Nichy 1958; Garton and Stickle 1980), yet these taxa groups did not comprise a major percentage of the commensal community in the latter half of the colonization period.

As previously mentioned, there was a significant oil effect on spat recruitment, however, this effect varied differentially between the June and September replicates. In June 2013, spat abundance was significantly greater on the oiled substrate than control. Previous research has indicated the presence of oil can lead to the creation of biofilms due to the bacterial degradation of hydrocarbons, which may act as a settlement cue (Cole and Knight-Jones 1939; Tamburri et al. 1992; McCoy and Brown 1998; Banks and Brown 2002). There was no significant difference in spat shell length between oil and control treatments, however, so perhaps the presence of hydrocarbon-related biofilms only enhances spat recruitment and not growth. In September, the abundance of live spat was significantly greater on control shells than on those that were soaked in oil. Furthermore, while spat steadily grew over time, the mean shell length was smaller in September compared to the values documented in June, perhaps indicating that certain seasonal or abiotic factors are more important for determining spat growth than are hydrocarbons, particularly in the absence of predators.

The short-term response to heavy oil contamination did have a measurable, albeit small, effect on both oyster commensal community structure as well as new oyster recruitment. Long-term monitoring shows that these effects are likely to diminish over time, and other factors such as salinity or seasonal variation may be more consistent drivers of oyster reef communities. Despite these findings, the ability of ecosystems and individual organisms to recover from the effects of oil may be further compromised by additional disturbances, especially in the Northern Gulf of Mexico where stressors like eutrophication and hypoxia from nutrient loading,

freshwater inputs, hurricanes, and habitat destruction are prevalent (Wells et al. 2004; Rabalais et al. 2007). Even a pre-adaptation to hydrocarbon exposure may not be powerful enough to withstand the synergistic effects of these anthropogenic stressors, especially in already threatened habitats such as oyster reefs.

Fortunately, restoration efforts can enhance oyster reefs and recover lost ecosystem services (Peterson et al. 2003a; Campbell 2004; Nelson et al. 2004; Piazza et al. 2005; Scyphers et al. 2011). This includes the commensal communities and the propagation of new oysters via the addition of any new hard substrate. In recent years, the focus on oyster reef restoration has shifted to include recovering lost ecosystem services; however, this has yet to include enhancement of commensal organisms, which are important food sources for higher trophic levels. While numerous studies have highlighted the importance of hard substrate for the recruitment of commensal macrofauna, few have looked at the efficacy of different substrate materials for enhancing these communities.

The substrate effects on taxa richness and diversity were inconsistent and varied depending on the time of year; however, OysterCrete significantly enhanced commensal organism abundance, compared to shell and limestone rocks, likely due to an increased amount of surface area and interstitial spaces than the other materials, although this was not quantified. The quantity and quality of available refuges will have a greater impact on the number of organisms a habitat can support than it will on the number of taxa. Differences in refuge availability could influence the diversity of the commensal community, depending on the size of and life history characteristics of certain species, as well as the size of the microhabitats created within or between substrates. For example, a reef will be dominated by polychaetes, small Xanthid crabs, and other epibenthic infauna if they are able to maneuver into the interstitial spaces better than

larger species, like skillettfish. While smaller interstitial spaces between substrates may prove favorable for some taxa by providing hiding spaces impenetrable by larger mobile predators, it may also lead to increased sedimentation, which could reduce the amount of available refuge space and make these organisms more susceptible to predation.

The structural arrangement as well as the material used can also determine the success of restoration projects. Bioengineered materials such as OysterCrete are advantageous in that they can be molded into a variety of shapes and sizes, which could allow them to withstand strong wave energies better than shell mounds. Furthermore, these composite materials often result in an outer surface high in rugosity, which increases available refuge and settlement space. Many bioengineered reefs are further enhanced with biological materials (commonly, cottonseed) to enhance oyster spat recruitment (Campbell 2004; Ortego 2006; Hall et al. 2009).

Despite the potential benefits of artificial reefs, differences between field sites resulted in greater differences in commensal community dynamics than did substrate material. The high salinity sites generally had increased abundance and taxa richness compared with the low salinity sites, which corresponds with previous work indicating that salinity is a major driver in benthic macrofauna distribution (Rosenberg et al. 1992; Brown and Stickle 2002; Hulathduwa et al. 2007). Additionally, the control high-salinity site generally had the greatest abundance of organisms, which suggests that there may be no long-term synergistic effects between hydrocarbon contamination and substrate materials, but further analysis of the trace hydrocarbons on the various materials is necessary to thoroughly examine this relationship.

While certain substrate materials and reef arrangements will provide more refuges than others, the availability of any hard substrate seems to be the most important determinant for colonization by commensal organisms (Humphries et al. 2011). Oyster reefs rival coral reef

habitats in terms of structural heterogeneity (Harding and Mann 1999), and it is this physical complexity that supports the niches of these macrofaunal organisms. Studies comparing live oyster clusters to disarticulated shell have indicated that the habitat complexity and refuge availability are more critical drivers of colonization than food resources provided by live oysters (Brietburg 1999; Tolley and Volety 2005; Humphries 2010). However, other ecosystem services provided by live oysters may further enhance the longevity of commensal organisms on oyster reefs. Conversely, the effects of hydrocarbon contamination on live oysters may negatively impact these commensal communities directly or indirectly via the decline of essential ecosystem services. The metamorphosis of artificial materials into a “living” reef with a high cover of adult oysters will likely facilitate the recovery of ecosystem services, regardless of the intended restoration goal.

The substrate materials were also analyzed for spat recruitment and growth. While the material type did not affect spat growth, the shell treatment significantly enhanced oyster spat recruitment in June 2013. In September 2013, however, spat abundance was greatest on the limestone rocks, although not statistically significant. These increases in spat abundance, however, may have been an artifact of site or seasonal differences as the oiled high-salinity site had the highest spat density across all substrate treatments in June, and the control low-salinity site had the overall highest spat abundance in September. These results correspond with previous work in which oyster larvae in coastal Louisiana were found documented to have settlement peaks in early and late summer, with later summer months producing the highest spatfall rates (Supan 1983). Site differences, although neither large nor consistent, also appeared to have a greater effect on spat growth than substrate material.

Restoration efforts along the northern Gulf of Mexico date back nearly 50 years (Furlong 2012) and a recent survey by La Peyre et al. (2014) reported that over half of the documented artificial reefs were created from rock-based materials including various forms of limestone and concrete, while bivalve shells were the second most commonly used materials, accounting for 20% of constructed reefs. Conservation managers must take into consideration numerous factors when restoring natural oyster reefs or designing artificial reef projects, including cost, resource availability, local conditions, and the desired end point for the stakeholders involved. Often, when finances or materials are a limiting factor, substrate materials will be spread thinly in an effort to maximize reef space per unit of materials. This tends to be an unfavorable option for commensal organisms and new oyster settlement alike as a thin layer of substrate is likely to sink into soft mud or silt, or be covered in sediment which often results in hypoxic conditions (Baker and Mann 1992; Soniat et al. 2004). As the production of new oysters is stunted, so are the subsequent ecosystem services they provide, including the creation of additional hard substrate.

Dense piles of dead oyster shell are another highly utilized design of oyster reef restoration. Dead oysters have been shown to be just as effective as live oysters in facilitating new oyster settlement as well as commensal communities (Plunket and La Peyre 2005; Tolley and Volety 2005). However, this strategy may also prove problematic as loose shell is susceptible to scattering if disturbed by high flow rates from waves, currents, and even boating activities (Lenihan 1999). In a recent survey by Brown et al. (2014), historic reefs were found to have a lower abundance of commensal organisms due to shell loss over time. Both the use of loose oyster cultch as well as thinly spread layers of any substrate material may result in an inefficient use of resources if the objective is to develop living and functional oyster reefs. Brown et al. (2014) also found that artificial reefs composed of rocks, which are denser than oyster shells, can

support the integrity of the reef over longer time periods, and therefore may be a more suitable material for maintaining ecosystem services.

The results from this study reveal that salinity will be a greater determinant for the success of new reef colonization, and should be considered in regards to placement of new artificial reef construction projects. Additionally, even if abundance or diversity are reduced at oil-contaminated sites following a spill event, this study provides support for the construction of artificial reefs projects in areas affected by future oil spills or other anthropogenic disturbances, as the availability of new hard substrate will facilitate colonization by macrobenthic invertebrates and fish.

Numerous studies comparing natural versus restored reefs, varying degrees of structural and vertical complexity, and artificial reef materials have resulted in somewhat conflicting results regarding the efficacy of these different variables on the enhancement of both sessile and mobile populations of reef inhabitants. There is a general consensus, however, that the sheer presence of a hard substrate will result in colonization by new oyster recruits and mobile nekton (Diehl 1992; Humphries et al. 2011; Brown et al. 2014), as long as the environmental conditions are favorable. If certain construction materials or arrangements do not facilitate noteworthy biological results in regards to commensal assemblages, perhaps the best solution for new artificial reef projects would be to utilize techniques that enhance some other aspect of oyster reef ecological functioning with the most cost-effective and durable resources available. In areas such as the northern Gulf of Mexico where oysters are likely to remain imperiled with the unpredictable yet prevalent likelihood of major disturbances from hurricanes, oil spills, and other anthropogenic impacts, restoration efforts will remain a critical solution for the continued sustainability of oyster reefs and their vital ecosystem services.

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APPENDIX

Table A.1. Identification of collected organisms based on taxonomic relationships.

Taxonomic Grouping	Taxonomic Level	Species Included	Common Name
Actinopterygii	Class	<i>Chasmodes bosquianus</i>	Striped Blenny
		<i>Gobiesox strumosus</i>	Skilletfish
		<i>Gobiosoma bosc</i>	Naked Goby
		<i>Ophichthus puncticeps</i>	Palespotted Eel
		<i>Opsanus tau</i>	Oyster Toadfish
Alpheidae	Family	<i>Alpheus heterochaelis</i>	Bigclaw Snapping Shrimp
Caenogastropoda	Order	<i>Bittium sp.</i>	
		<i>Epitonium sp.</i>	
		<i>Hydrobiid sp.</i>	
		<i>Texadina sphinctostoma</i>	Narrow Mouth Hydrobe
Hoplonemertea	Order	Nemerteans	
Mytilidae	Family	<i>Geukensia demissa</i>	Ribbed Mussel
		<i>Ischadium recurvum</i>	Hooked Mussel
Neogastropoda	Order	<i>Cantharus cancellarius</i>	
		<i>Nassarius acutus</i>	Sharp-knobbed Dog Whelk
		<i>Nassarius vibex</i>	Bruised Nassa
		<i>Stramonita haemastoma</i>	Gulf Oyster Drill
Nuculanidae	Family	<i>Nuculana acuta</i>	Pointed Nut Clam
Odontodactylidae	Family	<i>Odontodactylus scyllarus</i>	Peacock Mantis Shrimp
Ophiuroidea	Class	Brittle Stars	
Other Gastropoda	Class	<i>Acteocina canaliculata</i>	Channel Barrel Bubble
		<i>Nertina usnea</i>	Olive Nerite
		<i>Odostomia sp.</i>	
		<i>Turbonilla sp.</i>	
Paguroidea	Superfamily	<i>Clibanarius vittatus</i>	Striped Hermit Crab
		<i>Pagurus longicarpus</i>	Long-Armed Hermit Crab
Penaeidae	Family	<i>Farfantepenaeus aztecus</i>	Brown Shrimp
		<i>Litopenaeus setiferus</i>	White Shrimp
Peracaridae	Superorder	Amphipods	
		Isopods	
Polychaeta	Class	Polychaetes	
Porcellanidae	Family	<i>Pestrolisthes armatus</i>	Green Porcelain Crab
Portunidae	Family	<i>Callinectes sapidus</i>	Blue Crab
		<i>Callinectes similis</i>	Lesser Blue Crab
Styelidae	Family	<i>Styela plicata</i>	Sea Squirt

Table A.1 (Continued)

Veneroida	Order	<i>Macoma mitchelli</i>	Saltwater Clam
		<i>Mulinia sp.</i>	
		<i>Tagelus plebius</i>	Stout Razor Clam
Xanthoidea	Superfamily	<i>Eurypanopeus depressus</i>	Depressed Mud Crab
		Juvenile/Unidentifiable Xanthids	
		<i>Menippe adina</i>	Gulf Stone Crab
		<i>Panopeus herbstii</i>	Black-Clawed Mud Crab
		<i>Panopeus obesus</i>	Salt Marsh Mud Crab
		<i>Panopeus simpsoni</i>	Oystershell Mud Crab
		<i>Rhithropanopeus harrisi</i>	Dwarf Crab

VITA

Jenessa Kay was born in January 1986 in Miami, Florida. From an early age, she was always interested in marine biology and natural resource conservation. She attended the University of North Carolina at Chapel Hill and graduated in 2010 with a Bachelor of Arts in Biology and a minor in Marine Sciences. During her summers as an undergrad she held an internship at the UNC Institute of Marine Sciences where she assisted with research projects examining the effect on sea level rise on secondary productivity of estuarine ecosystems. She was also given the opportunity to conduct an independent research project observing the intraspecific interactions of crabs, which contributed to a publication in *Marine Ecology Progress Series* in 2010.

She has also worked as a research technician at the Dauphin Island Sea Lab, served as a biological consultant to research the impacts of the *Deepwater Horizon* oil spill on coastal Louisiana through NOAA's Natural Resource Damage Assessment Program, and volunteered at the ARCHELON Sea Turtle Protection Society of Greece. During her time at LSU she was the teaching assistant for the Marine Communities Laboratory, including teaching the course abroad in Juneau, Alaska. She served as the BioGrads Social Chair and presented her research at several scientific conferences, including the Benthic Ecology Meeting, the Coastal and Estuarine Research Federation Biannual Conference, the Graduate School Symposium, and the BioGrads Symposium.

She is the daughter of James and Janice Kay, and is the sister of PFC Chelsea Kay, US Army. In her free time she enjoys outdoor adventure, traveling, cooking, playing with her dogs, cat, and rabbit, and is an avid sports fan.